THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME LXV

BALTIMORE, MD.
1923

Medical Lib.
JUN 6 1923

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXV—No. 1
Issued June 1, 1923

BALTIMORE, U. S. A.
1923

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

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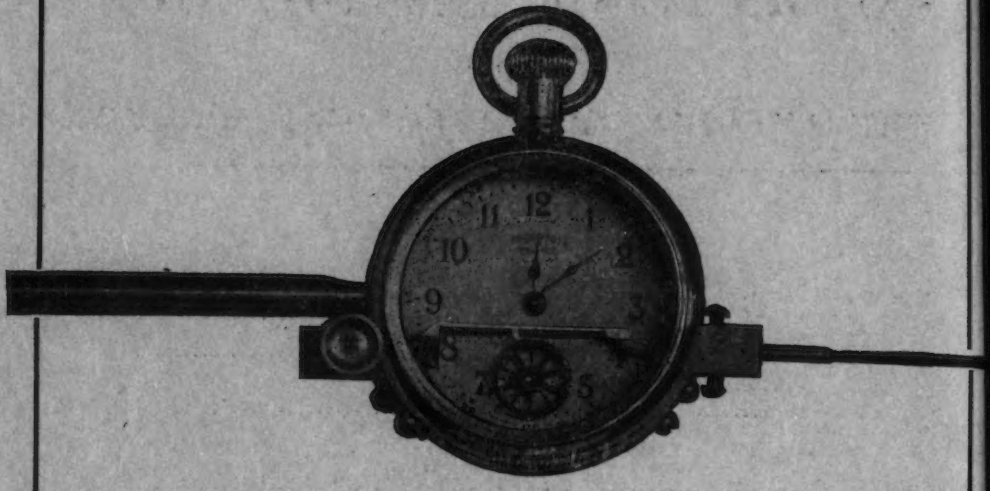

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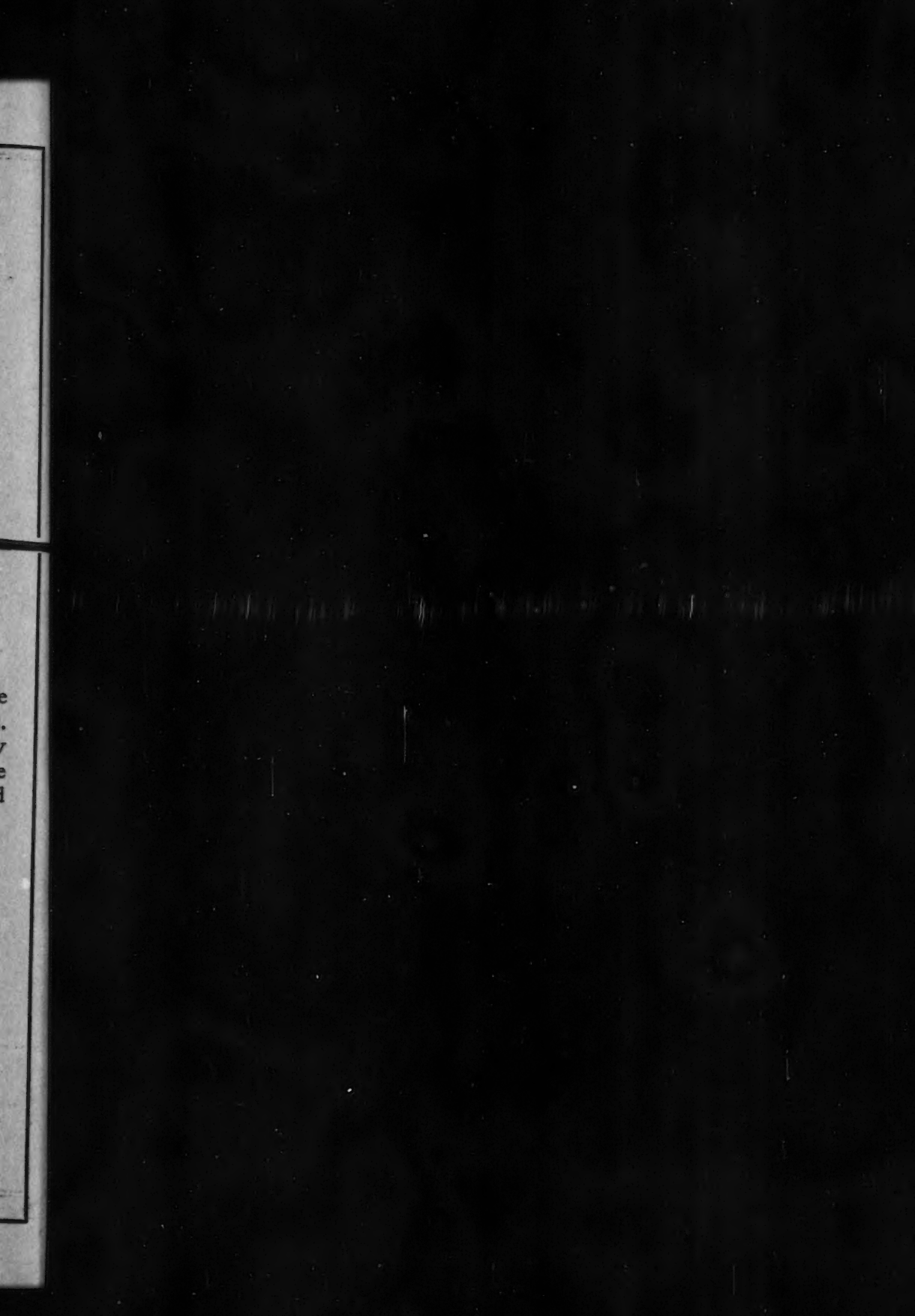

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

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THE EFFECT OF EXPERIMENTAL RICKETS UPON THE WEIGHTS OF THE VARIOUS ORGANS IN ALBINO RATS

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Received for publication February 3, 1923

The anatomical lesions in rickets have hitherto been sought chiefly in the skeleton, although an involvement of other organs, especially of the liver, spleen and ductless glands, has often been suspected. Since the effects upon organs are frequently evident by changes in weight, it seems desirable to review systematically the changes during experimental rickets from this point of view.

Material and methods. For the present study, 118 albino rats were used. Of these, 37 are normal controls, 3 to 16 weeks of age, and 22 to 135 grams in body weight. They had been fed chiefly whole-wheat bread and whole milk, supplemented by green vegetables, occasional meat, etc. Out of a large number of rats which had been autopsied (for other purposes) in the Institute of Anatomy, these were selected as controls, corresponding as nearly as possible in body length to the rats used in the present experiments.

The present test rats are 81 in number, ranging from 33 to 147 days of age, and 22 to 132 grams in weight. Most of them are between 2 and 3 months of age and between 30 and 75 grams in body weight, being usually somewhat retarded in comparison with the normal for corresponding age. For these test rats we are indebted to Prof. J. F. McClendon, who had fed them various diets to produce experimental rickets ('22). The experiments usually began when the rats were about one month old. In most cases patent (white) wheat flour formed the basis of the diet, as recommended by Sherman and Pappenheimer ('21), supplemented by casein or lactalbumin, various salt

mixtures, and variable amounts of yeast and spinach. In a few cases mixtures of starch, cotton seed oil, casein and lactalbumin replaced the flour. In general, the diets deficient in phosphorus and vitamins (especially A) seemed to be more effective in the production of rickets.

After x-ray examination, the rats were killed by ether or chloroform, and were autopsied in a uniform manner. The general appearance, body weight, body length (nose-anus) and tail length were first recorded. The head was then separated just behind the cranium and in front of the larynx. It was then weighed, the trunk in the meantime being suspended by the tail to drain the blood (unmeasured). Then the various organs were carefully removed, being placed in a moist chamber until weighed, to prevent evaporation. The thyroid gland was not weighed in these rats, but was preserved for histological study. The spinal cord also was weighed in only a few cases, and is not included in the data considered.

Weights for the entire skeleton and the musculature were obtained in some cases as follows. After removal of the integument (which was cleaned of subcutaneous fat and weighed), and of all other organs, together with adherent fat, etc., the skeleton and musculature were weighed together. Then the musculature, including the tendons, was carefully dissected off, leaving the entire skeleton with ligaments intact, so far as possible. This ligamentous skeleton was then weighed, and the weight of the musculature obtained by difference. In some cases, small portions (weighing about 0.1 gram) of the costochondral joints and upper extremity of the tibia were removed for histological study. The ligamentous skeleton was then macerated for about 1 hour in 1 per cent aqueous solution of "Gold dust washing powder," said to contain sodium carbonate, 45 per cent; soap powder, 30 per cent; water 25 per cent (Donaldson and Conrow, '19). The skeletons were macerated at a temperature just below boiling point. The adherent remnants of the ligaments and periosteum were then removed with a small brush, under water, and the remaining cartilaginous skeleton was weighed moist, after removal of the adherent water by filter paper. Finally the cartilaginous skeletons were all dried together for about 3 weeks in open vials in an oven electrically heated to about 95°C., and the dry weight of each skeleton was obtained.

Having thus obtained the weights of the various systems and organs in both test rats and controls, the question of a method of comparison arose. This is always a difficult problem in experimental work of this kind, in which the conclusions are frequently unjustified, on account

of insufficient data, inadequate controls and improper methods of comparison. Especially in young animals, the rapid shifts in normal size (relative and absolute) and in structure are likely to make comparisons misleading. In the albino rat we fortunately have a species which has been fairly well standardized for body size and weights of the principal viscera. These have been published in convenient form by Donaldson ('15), together with a good method of comparison for experimental work. The weight of each organ is compared with the norm for corresponding body length (the most satisfactory basis of comparison). The percentage deviation from the norm for each individual organ is then calculated, for both test rats and controls. A comparison of these percentage deviations will indicate whether the apparent changes in the test rats are due to the conditions of the experiment, or are found also in the (presumably normal) controls.

While Donaldson's tables include most of the organs considered, it was necessary to supplement them with data from Jackson and Lowrey ('12) for the weights of the head, integument, musculature and ligamentous skeleton; from Jackson ('13) for the stomach and intestines with contents; from Donaldson and Conrow ('19) for the fresh cartilaginous skeleton; and from Lowrey ('13) for the dry skeleton. In the case of these supplementary data, graphs were drawn for the weight of each organ according to body weight instead of body length (which was not available), and comparisons made as mentioned above. Since in the present experiments the body length remained fairly constant in proportion to the body weight, however, the results of comparison should be very similar, whether body length or body weight is used as the basis.

In order to reveal possible differences in the effects according to the stage or severity of the disorder, the percentage deviations from the norm were averaged in five groups, as shown in tables 1 and 2. These groups were arranged on the basis of the preliminary x-ray examination (especially the thickness of the upper tibial epiphyseal cartilage), and the gross skeletal lesions as follows: *a*, 37 normal controls; *b*, 27 apparently normal, although they had been upon some form of experimental diet; *c*, 19 slightly rachitic (slight costochondral enlargements); 19 moderately rachitic (definite costochondral enlargements, with slight softening and mild deformity of thoracic and limb skeleton); *d*, 16 severely rachitic (marked costochondral enlargements, with pronounced softening and extreme deformity of the skeleton, especially of the thorax and limbs).

TABLE 1

Rats with experimental rickets and controls. Average percentage deviation from norm for organ weights

The corrected data (in parenthesis) for the test rats were obtained by subtracting the average deviation in the corresponding controls.

		TEST RATS ON EXPERIMENTAL DIETS			
	37* normal controls	27* apparently normal	19* slight rickets	19* moderate rickets	16* severe rickets
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Body weight	+ 8.9	+ 9.8(+0.9)	+ 8.3(−0.6)	+12.4(+3.5)	+ 4.6(−4.3)
Tail length	− 5.3	0(+5.3)	− 4.6(−0.7)	+ 3.3(+8.6)	+ 4.4(+9.7)
Head weight	− 2.5	+ 1.9(+4.4)	+ 4.7(+7.2)	+ 8.5(+11.0)	+ 2.5(+5.0)
Integument	−10.0	− 2.2(+7.8)	−30.0(−20.0)	−24.1(−14.1)	−22.1(−12.1)
Lig. skeleton	−13.2	+ 5.9(+19.1)	−13.2(0)	+ 1.8(+15.0)	+ 2.6(+15.8)
Cart. skeleton	+24.3	+ 3.8(−20.5)	+22.6(−1.7)	+21.0(−3.3)	+17.0(−7.3)
Cart. skeleton (dry)		+27.3	− 7.3	−19.1	−18.5
Musculature	+ 4.4	+ 7.4(+3.0)	+ 3.2(−1.2)	− 3.4(−7.8)	− 7.8(−12.2)
Submaxillary glands	+43.3	+21.9(−21.4)	+58.3(+15.0)	+95.2(+51.9)	+92.3(+49.0)
Brain	+ 1.8	− 2.1(−3.9)	− 2.3(−4.1)	− 1.1(−2.9)	− 2.6(−4.4)
Eyeballs	+14.5	+15.5(+1.0)	+19.6(+5.1)	+28.8(+14.3)	+40.6(+26.1)
Thymus	+ 3.4	−30.1(−33.5)	−33.3(−36.7)	−52.8(−56.2)	−66.5(−69.9)
Heart	+ 8.7	+23.5(+14.8)	+17.1(+8.4)	+28.8(+20.1)	+26.3(+17.6)
Lungs	+ 7.9	+13.4(+5.5)	− 0(−7.9)	+16.8(+7.9)	+ 6.1(−1.8)
Liver	−22.0	− 2.1(+20.9)	+14.1(+36.1)	− 8.9(+13.1)	−27.7(−5.7)
Spleen	+53.2	+13.7(−39.5)	+27.7(−25.5)	+61.9(+8.7)	+37.7(−15.5)
Stomach—intestines (empty)	−25.0	−43.0(−18.0)	−34.0(−9.0)	− 7.7(+17.3)	−48.7(−23.7)
Stomach—intestines (+ contents)	− 0.1	+12.9(+13.0)	+33.4(+33.5)	+14.7(+14.8)	+25.6(+25.7)
Suprarenals	− 2.3	+42.4(+44.7)	+42.0(+44.3)	+45.8(+48.1)	+62.0(+64.3)
Kidneys	+ 9.5	+42.8(+33.3)	+54.5(+45.0)	+57.6(+48.1)	+51.2(+41.7)
Ovaries	+13.2	+12.5(−0.7)	+36.8(+23.6)	+41.3(+28.1)	+ 4.7(−8.5)
Testes	+15.8	+55.8(+40.0)	− 1.7(−17.5)	+ 4.0(−11.8)	+21.2(+5.4)
Epididymides	+11.8	+35.1(+23.3)	+ 5.4(−6.4)	+44.1(+32.3)	+30.6(+18.8)
Hypophysis	+15.8	−10.9(−26.7)	−12.2(−28.0)	− 8.4(−24.2)	− 1.4(−17.2)

* In the case of the integument, skeleton, musculature, empty stomach-intestines, submaxillary glands and gonads, the number of observations is considerably less, as explained in the text.

On account of individual variations, there was naturally difficulty in grouping some of the cases, but the final result probably represents approximately the degree of severity of the rachitic lesions. Since the diagnosis of rickets is always uncertain upon the basis of gross appear-

TABLE 2

Cartilaginous skeleton of rats with experimental rickets. Individual data

RAT NUMBER	AGE	BODY WEIGHT	MOIST CARTILAGE SKELETON	DRY CARTILAGINOUS SKELETON		
				Weight	Per cent of fresh cartilaginous skeleton	Compared with norm
Apparently normal rats						
	days	*grams*	*grams**	*grams*	*per cent*	*per cent*
25.1	63	70	6.9	2.657	38.5	+ 6
25.2	67	75	6.8(+0.1)	2.978	43.8	+10
25.3	65	91	8.1	3.704	45.7	+14
25.4	65	86	8.9(+0.1)	3.706	41.6	+24
25.5	63	93	9.0	4.485	49.8	+37
25.6	68	64	7.1(+0.1)	3.657	51.5	+66
27.1	61	71	8.1	3.347	41.2	+34
Slight rickets						
27.3	61	74	6.2	1.974	31.8	−24
28.2	86	90	8.7	3.622	41.6	+13
29.3	72	66	6.5(+0.1)	2.045	31.5	−11
Moderate rickets						
27.4	58	72	6.4	1.392	21.8	−44
28.1	84	84	8.0	2.393	29.9	−20
29.1	70	71	6.8(+0.1)	2.654	39.0	+ 6
Severe rickets						
27.5	58	59	5.0	1.603	32.1	−20
29.2	73	71	6.5(+0.1)	2.069	31.8	−17

* In some cases the weights do not include a small piece removed for histological examination (indicated in parenthesis).

ances alone, microscopic examination of the stained sections (from material with or without decalcification) was made, which corroborated the diagnosis. Although there are marked variations in histological structure, the characteristic osteoid tissue and other irregularities

occur in the rachitic skeleton, corresponding in general to the recent descriptions by Pappenheimer, McCollum, Park, Shipley and their co-workers, using similar methods.

Unfortunately the weights for all of the organs were not secured in all cases. Thus complete data for the skeleton were available in only 15 test rats, as shown in table 2. Other organs in which the number of observations is incomplete are as follows:

ORGANS	NUMBER OF OBSERVATIONS IN			
	Apparently normal	Slight rickets	Moderate rickets	Severe rickets
Integument	16	9	14	11
Musculature	9	4	7	5
Submaxillary glands	24	13	8	7
Empty stomach and intestines	6	3	4	1
Ovaries	10	5	7	2
Testes	15	9	9	8
Epididymides	14	5	6	5

For the other organs the data are complete, or nearly so, in each case. Even in these organs, however, the numbers in each group are too small to justify a statistical analysis which is highly desirable. The normal variation in the weights of the organs in the rat was studied by Jackson ('13). The present data allow merely a preliminary study, and the results are to be regarded as suggestive rather than conclusive.

OBSERVATIONS (table 1): *Body weight.* The body weight (compared with the norm for corresponding body length) averages for all groups slightly above the norm. The normal controls themselves apparently average 8.9 per cent above the norm, and if this figure is subtracted from the data for each test group, as indicated in parenthesis, it is seen that the body weight is apparently nearly normal in relation to body length. To a certain extent, however, this appearance is deceptive, since an increase in the intestinal contents (as will be shown later) masks a certain amount of loss in body weight in the test rats.

The *tail length* likewise shows no marked change in the test rats, although there is an evident tendency to relative elongation in the groups with moderate and severe rickets.

The *head weight* appears slightly above normal in all the test groups, but the increase is small and of doubtful significance, especially in the severely rachitic group.

The *integument* in the "normal controls" averages 10 per cent below the norm for body length. In the test groups (excepting the "apparently normal"), the integument is still more subnormal in weight, though not progressively so, varying from 20 per cent below in the slightly rachitic to 12 per cent below in the severely rachitic. The decreased weight is possibly due to loss in fat.

Skeleton. The averages for the various groups are shown in table 1. In view of the small number of observations and the marked individual variations, as shown in table 2, conclusions must be correspondingly guarded.

The *ligamentous skeleton* in the test groups shows no significant deviation in weight from the norm for corresponding body length. Since the "normal controls" average 13 per cent below the norm, however, the corrected data for the test rats appear correspondingly higher. But this apparent increase occurs also in the "apparently normal" test group, and is of somewhat doubtful significance.

The moist *cartilaginous skeleton* appears considerably above the norm in the rachitic groups; but this is also true for the "normal controls." These apparent differences are probably due to a difference in technique by which the skeletons for the norm were prepared (by Donaldson and Conrow). It is therefore doubtful whether there is any significant change in the weights of the moist cartilaginous skeleton. The individual data are given in table 2.

For the *dry cartilaginous skeleton*, however, the results are quite different. No data for the dry skeleton were available in the "normal control" group. In the "apparently normal" test group, the dry skeleton averaged 27 per cent above the norm. In the slightly rachitic rats it averaged 7 per cent below the norm, and in the moderately and severely rachitic about 19 per cent below. The marked individual variation is shown in table 2. Thus the weight of the dry skeleton in the "apparently normal" group ranged from 6 to 66 per cent above norm; in the slightly rachitic from 13 per cent above to 24 below; in the moderately rachitic from 6 per cent above to 44 below; and in the two severely rachitic 17 and 20 per cent below the norm. Since the moist skeleton is usually nearly normal in weight, these data for the dry substance indicate an extremely variable deficit in dry substance (especially in calcareous material).

The data for the *musculature* indicate a slight but progressive loss in weight for the three rachitic groups, amounting to about 12 per cent subnormal in the severely rachitic.

The *submaxillary glands* appear markedly (43 per cent) above norm in the "normal controls," possibly due to the inclusion of associated lymph nodes. Even deducting this figure from the averages for the test groups, however, there remains an apparent increase of 15 per cent in the slightly rachitic, 52 per cent in the moderately rachitic and 49 per cent in the severely rachitic groups.

The *brain* is remarkably uniform in weight in all groups, with very slight individual variability. It appears below the norm in all the test groups; but the difference is very slight (less than 5 per cent, even in the corrected groups), and shows no progressive change, so it is of doubtful significance.

The *eyeballs*, on the other hand, appear above the norm in all groups. Even when the 14.5 per cent excess for the "normal control" group is deducted, the corrected data for the test groups show a progressive increase up to 26 per cent above for the severely rachitic group.

The *thymus* averages slightly above the norm in the "normal control" group, but shows a progressive decrease in the test groups, reaching about 70 per cent subnormal weight in the severely rachitic group. This may appear remarkable when it is recalled that the body weight-length ratio is nearly normal, but on the other hand nearly all of the test rats were subnormal in weight according to age, indicating a retardation in growth.

The *heart* weight averages above the norm in all groups, even slightly (8.7 per cent) in the "normal controls." Deducting this figure gives corrected data indicating a hypertrophy in the test groups, even in the "apparently normal," but most marked (18 to 20 per cent) in the moderately and severely rachitic.

The *lungs* show some irregularities in weight, but apparently no significant deviation from the norm.

The *liver* appears irregular in weight. Even the "normal controls" average 22 per cent below the norm. (Donaldson's norm based upon Hatai's data is probably somewhat high.) When the data for the test groups are correspondingly corrected, it would appear that the liver is above normal in weight in the earlier stages, 21 per cent above in the "apparently normal" and 36 per cent in the slightly rachitic; decreasing to 13 per cent above normal in the moderately rachitic and 6 per cent subnormal in the severely rachitic.

The *spleen* is an organ notably variable even in normal weight. In our "normal control" group it averages 53 per cent above Donaldson's norm (which appears too low, because the largest spleens were excluded

in Hatai's data upon which this norm is based). Deducting this figure to obtain the corrected data for the test groups, the spleen averages about 40 per cent subnormal in the "apparently normal" group, 26 per cent subnormal in the slightly rachitic, 9 per cent above normal in the moderately rachitic and 16 per cent subnormal in the severely rachitic. These figures are so irregular that their significance is very doubtful.

The *empty stomach and intestines* appear below the norm in weight in all groups, even in the "normal controls" (−25 per cent). In the corrected data, the test groups still appear subnormal, excepting the moderately rachitic, which averages 17 per cent above. The data for the empty canal are somewhat inconclusive, on account of both the scanty data and the difficulty in obtaining a uniform technique; but on the whole they appear to indicate a tendency to subnormal weight in the rachitic rats.

In the *stomach and intestine with contents*, on the other hand, there is a marked increase in weight, averaging 13 per cent above normal in the "apparently normal" group, and 15 to 34 per cent in the rachitic groups. Since the empty canal is usually subnormal in weight, the increase is clearly due to excessive contents. The distention of the intestines with liquid as well as gas is often strikingly apparent in the rachitic rats. In extreme cases, the intestinal contents may equal 20 per cent or more of the body weight, which (as above mentioned) introduces a possible error of considerable importance in interpreting the gross body weight. Normally, the intestinal contents usually form less than 10 per cent of the gross body weight.

The *suprarenal glands* appear nearly normal (−2.3 per cent) in weight in the "normal control" group, but are markedly above normal in the test groups. The increase appears slightly progressive in character, the (corrected) weights appearing 45 per cent above in the "apparently normal" group, 44 per cent in the slightly rachitic, 48 per cent in the moderately rachitic, and 64 per cent in the severely rachitic group. Although there is a sex difference in the weights of the suprarenal glands (larger in the female rat), an examination showed the increased weight to be similar in both sexes, so the data are not separated in the table.

The *kidneys* average slightly (9.5 per cent) above the norm in the "normal control" group, but very much more in the test groups. The corrected data indicate a weight 33 per cent above for the "apparently normal" group, 45 per cent for the slightly rachitic, 48 per cent for the moderately rachitic and 42 per cent for the severely rachitic. The in-

crease is therefore fairly progressive for the various stages, excepting the last.

The *ovaries* (corrected data) appear considerably above normal in the slightly and moderately rachitic groups, but slightly subnormal in the severely rachitic. The *testes* also appear irregular, their deviations tending to be opposite to those for the ovary in corresponding groups. The *epididymides* apparently tend to hypertrophy in the moderately and severely rachitic groups (32 and 19 per cent above normal). On account of the small number of observations and the great physiological variability in the weights of the gonads, however, no definite conclusions can be drawn from these data.

The *hypophysis*, however, shows a fairly uniform tendency to subnormal weight. This affects both sexes, although they are not separated in the tabulation of the results. The "normal controls" averaged 16 per cent above the norm. The test rats (corrected data) show an average of 27 to 28 per cent subnormal in the "apparently normal" and slightly rachitic groups, but less marked (24 and 17 per cent subnormal) in the moderately and severely rachitic groups. The subnormality therefore appears greatest in the earlier stages.

Discussion. Although the literature on rickets is enormous, we find very little quantitative data for comparison with the present findings. For human rickets, the frequent enlargement of the liver and (especially) the spleen was noted by Whistler (1645) and by many subsequent observers; but the significance of this hypertrophy has been much disputed, according to reviews of the earlier literature by von Starck ('96) and Sasuchin ('00). Stoeltzner ('03) could find no significant visceral changes in rickets, with the possible exception of hydrocephalus. Vincent ('04) states that the lesions involve not only the bones, "but also implicate the brain, liver, spleen, muscles, and, indeed, every organ may be affected to a greater or less extent." The enlarged head is said to be due to thickening of the cranial bones, the facial skeleton being arrested in growth. The muscles are poorly developed. The spleen is moderately enlarged in the progressive stage, but not permanently. The liver is less frequently and less markedly enlarged.

Cheadle and Poynton ('07) agree that in human rickets the enlargement of the head is due chiefly to cranial thickening, the capacity being normal (except in hydrocephalus). The muscles are wasted; the liver usually normal in size, rarely enlarged; the spleen also sometimes enlarged. The skin may appear normal, except for anemia; the subcutaneous fat is variable, sometimes abundant, sometimes scanty. The heart appears variable, but probably shares in the general malnutrition.

Stoeltzner ('04, '08) claimed that the suprarenal glands are atrophied in rickets, but this was not confirmed by Cattaneo ('05). Stoeltzner ('09) also supports the theory of a specific regressive dystrophy of the musculature in rickets, including an atrophy of the smooth muscle in the intestinal wall (which results in meteorism). The frequent enlargement of the spleen is not considered essential. The thymus was found hypertrophied by du Castel ('08) and by Marfan ('22). Lehnerdt ('10) concluded (p. 134) that "Bei Rachitis ist die Knochengewebemasse als ganzes in der Regel nicht vermindert, sondern in ähnlichen Mengen vorhanden wie in der Norm, meist ist sie sogar in erheblichem Masse vermehrt."

In experimental rickets, E. Voit ('80) compared the organ weights of a normal puppy (body weight 4510 grams) with another of the same age and litter, which appeared markedly rachitic after a calcium-poor diet of extracted meat plus lard and distilled water (body weight 4710 grams). Voit's table of absolute and relative weights shows no significant difference between the two puppies in the weights of the skin, adipose tissue, muscles, bones (fresh), cartilage, brain, spinal cord, and empty stomach-intestine. The following organs appear larger in the test dog than in the control, as indicated (in parenthesis) by the percentage of the body weight formed by each organ in test and control, respectively: lungs (0.97–0.89 per cent); liver (5.45–4.74 per cent); heart (0.83–0.77 per cent); genito-urinary organs (kidneys and bladder?) (1.24–1.01 per cent); pancreas, spleen and thymus (1.07–0.42 per cent). The increases are thus relatively small, except for the last group (unfortunately weighed together). The weights of the dry skeleton indicate a loss in the rachitic puppy, with a water content of 71.9 per cent in comparison with 64.9 per cent in the control.

Dibbelt ('09) gives weights of several organs in 5 puppies, one normal and 4 rachitic (on calcium-poor diet of horse-meat and starch). Unfortunately only one of the test puppies (no. VI, 4995 grams) is near enough in weight to the control (no. IV, 6000 grams) to make even rough comparison possible. Relative to body weight, there is in these two cases apparently no significant difference in the weight of the brain, spleen and kidneys. The heart is absolutely as well as relatively larger in the rachitic puppy, weighing 43 grams, while only 39 grams in the larger control. The same is true for the moist humerus (28.5 grams in test puppy, 23.9 grams in the control); the dry humerus, however, weighs 13.0 grams in the rachitic, 9.8 grams in the control. The lungs appear atrophic in the rachitic puppy, weighing 35.5 grams, in comparison with 72.0 grams in the control; likewise the liver (116.5 to 203 grams).

In the more recent work on experimental rickets in rats by methods similar to those used in the present study, Shipley, Park, McCollum and Simmonds ('21) note incidentally that the spleen is found atrophic in some rats, more or less enlarged in others. The thymus is atrophic. McCollum, Simmonds, Shipley and Park ('21) state that in rachitic rats the spleen is frequently greatly enlarged, the thymus and gonads atrophic. McCollum, Simmonds, Kinney, Shipley and Park ('22) find the thymus atrophied in the rachitic rats. The spleen is enlarged in some cases, but usually not above normal.

Comparing our results with the foregoing, we may conclude that our findings as to the skeletal weights, fresh and dry, are in substantial agreement with the statements of previous observers in both human and experimental rickets. Likewise as to the atrophy of the thymus (excepting du Castel and Marfan), and the irregularity of the spleen. Like Cattaneo, we fail to confirm Stoeltzner's claim of a suprarenal atrophy in rickets, finding a hypertrophy instead. Although there is a slight tendency to agreement, we fail to find in the rats a pronounced atrophy of the musculature or a marked increase in the size of the head, both of which are considered characteristic for human rickets. Our observation of a cardiac hypertrophy in the rat agrees with Voit and Dibbelt for rachitic puppies, but has not been noted in human rickets. Similarly a hypertrophy of the kidneys, as found in our rats, appears also in Dibbelt's puppy, but not in Voit's. The integument appears variable in human and canine rickets, with no definite tendency to atrophy as found in our rats. The lungs were slightly irregular in weight with little change in our rats; in puppies, Voit found a slight apparent increase, but Dibbelt a decrease in weight. The liver also appears variable in weight, both in human and in animal rickets. As in our brain weights for the rat, no significant difference appears in the data of Voit and Dibbelt for rachitic puppies. Our data do not confirm the atrophy of the gonads found in rachitic rats by McCollum, Simmonds, Shipley and Park. No data for comparison were found concerning the weights of the hypophysis, stomach and intestines, eyeballs and submaxillary glands.

In interpreting the results of the present experiments, we must remember that even where the changes are unquestionably significant it does not necessarily follow that they are essential to rickets. They may be due to variations in the diet which have little or nothing to do with the production of the characteristic rachitic lesions. This may explain some of the marked changes in certain organ weights of the

"apparently normal" group. Similarly in human rickets some of the changes may be due to "complications." On the other hand, it is equally possible that some of these early changes in experimental rickets are due to the rachitic factors, which might cause unsuspected changes in organ weights before the typical rachitic lesions appear. Some of the changes, however, such as atrophy of the thymus and hypertrophy of the suprarenals, occur also during inanition and other nutritional disorders. Further investigation is therefore necessary before the significance of the changes in organ weight during experimental rickets can be interpreted.

SUMMARY

We may group the various organs and parts of the body, according to apparent changes in weight during experimental rickets in our rats, into 3 groups. The first group shows a definite decrease in weight. This group includes the integument, hypophysis, dry skeleton, empty stomach and intestines, and especially the thymus. The average decrease is regularly progressive in the case of the thymus and dry skeleton, although marked individual variations occur.

The second group, which shows an increase in weight, includes the eyeballs, heart, gastro-intestinal contents, and especially the submaxillary glands, kidneys and suprarenal glands. The average change appears fairly progressive in all except the heart and gastro-intestinal contents.

The third group includes those in which the apparent changes are slight or irregular. This group includes the head, ligamentous and (moist) cartilaginous skeleton, musculature, brain, lungs, liver, spleen, ovaries, testes and epididymides. The head, however, shows a slight tendency to increase and the musculature to decrease in weight. The liver averages above normal in the earlier stages, decreasing later. The gonads and spleen are very irregular in weight, the latter usually subnormal.

Only very scanty and indefinite data for comparison are found in the literature, but our findings appear to be in general agreement with those of previous observers on human and animal rickets as to the weights of some organs, including the fresh and dry skeletal weights, thymus (in rats), spleen, heart, liver, lungs and brain. To some extent this applies also to the head and musculature, but for other organs our results are in disagreement with those of previous workers, or there are no data available for comparison.

Great caution is necessary in interpreting these changes in organ weights, even where the changes are unquestionably significant, as they may be due to factors other than those essential to the production of rickets.

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DOES ADRENALIN AFFECT THE METABOLISM OF THE SURVIVING SKELETAL MUSCLES OF THE FROG?

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Received for publication February 9, 1923

The work to be reported upon here has to do with the effect of adrenalin upon the total acid production and also the carbon dioxid production of surviving skeletal muscles of the frog. Since it was begun, somewhat over a year ago, papers have appeared by Martin and Armitstead (1) and also by Hutchinson (2) which deal with these same problems.

Martin and Armitstead concluded from their work that adrenalin increases the total acid production or, as they also term it, the metabolism of muscles. Once or twice in the body of their paper they seem to imply that carbon dioxid is responsible for this increase. But they are much safer in their general conclusion because, as Hutchinson pointed out, their method—the indicator method of Haas (3)—did not permit them to distinguish between carbon dioxid and any other acids that might be produced at the same time.

Hutchinson made this distinction and, by means of the Osterhout apparatus and technique (4), studied carbon dioxid production alone. Her results led her to conclude that strong solutions of adrenalin (0.002 to 0.003 per cent) cause a depression in the carbon dioxid production of muscles, which is followed by a return to normal—probably due to the oxidation of the adrenalin; weaker solutions, on the other hand, produce a peculiar rhythmic effect in which the rate of production of carbon dioxid falls, rises and then falls and rises again.

Comment upon these two papers will be made later.

EXPERIMENTS AND DISCUSSION. Two forms of adrenalin were employed in this work—the 1:1000 adrenalin chloride solution and the adrenalin powder, which are prepared by Parke, Davis & Co.

A specially prepared Ringer solution was used in all of the experiments which are to be described. This contained only sodium, potassium and calcium chlorides, in the usual proportions for frog tissues,

but no bicarbonate. As thus prepared, it had a pH[1] value slightly less than 7, i.e., it was practically neutral. Reference to "Ringer solution" throughout this article, unless otherwise qualified, will always be to this specially prepared and practically neutral solution of the three chlorides.

The experiments were carried out at ordinary room temperatures which varied from 18° to 22°C. in winter to 25° to 28°C. during the summer. At no time were the temperature changes, during a single experiment, great enough to have significant effects upon the rate of carbon dioxid production.

I. The effect of adrenalin upon carbon dioxid production. It is a pleasure to acknowledge my indebtedness to Professor Osterhout for the suggestion that I use his apparatus (4) in studying the effect of adrenalin upon the carbon dioxid production of muscles. This I have done, but with the slight change shown in figure A. This modification suggested itself because it was found that opening the original apparatus for any purpose—as for adding some substance whose effect on the rate of respiration is to be studied—temporarily destroys the carbon dioxid equilibrium within the previously closed system, on which the delicacy and accuracy of the method depends. Also, such a change of solutions, in itself, introduces a further disturbing factor. For even if precaution has been taken to have the solutions of the same hydrogen ion concentration and the same buffer action, the newly introduced solution will require some time to come into equilibrium with such carbon dioxid tensions as previously existed within the closed system. Such disturbances obscure the true results and, in my experience, some ten or fifteen minutes may be required, upon continuing the experiment, to obtain trustworthy determinations.

These difficulties were encountered very early in the work. Hutchinson, who also used the Osterhout technique, had similar trouble, because she says that "in order to avoid effects due to the admission of laboratory air and to absorption of CO_2 by the solution of adrenalin it was decided to discard the readings made during the first ten minutes after the solution of adrenalin was added."

[1] It must be mentioned here that the pH values given in this paper may not be absolutely correct. They were all determined by means of indicators and none of the standards were checked electrometrically. But all that was needed in this work was to have solutions of the *same* hydrogen ion concentration and this could be accomplished, with all necessary exactness, by simply matching their colors by means of indicators.

It seemed possible, however, that if adrenalin had any effect upon tissue metabolism it might be an immediate one which would be lost, or overlooked, by such a procedure. At all events, it was thought safer to use a method which avoided such disturbances altogether and which would make it possible to recognize any immediate variations in the carbon dioxid production, or none at all, as the case might be. This was done as follows.

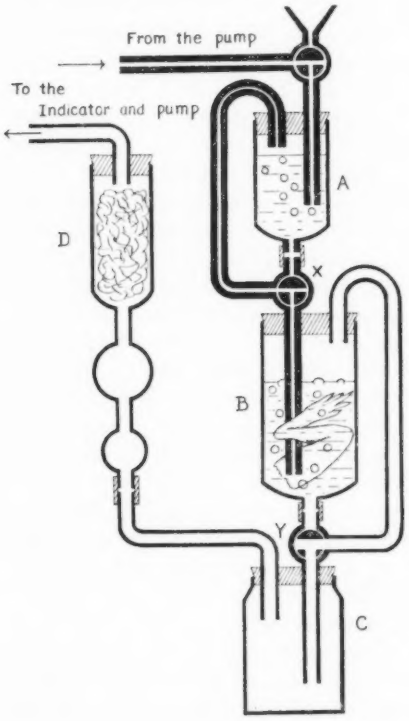

Fig. 1 A

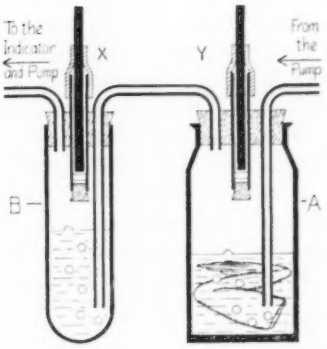

Fig. 1 B

The apparatus and its use: The vessel *B* (fig. 1 A) contains the tissue whose respiration is being studied and, at the beginning of the experiment, the control solution. *A* contains the solution whose effect is to be tested. These two chambers, as well as *C*, are in direct communication, so that air driven by the pump of the apparatus (not shown in the figure) circulates freely through all of them. *D* is a tube containing

a small cotton plug, which it was found useful to have in the circuit, for these experiments, in order to prevent any froth from being blown over into the indicator. The use of the apparatus may be best learned from the description of a typical experiment.

The work of Fletcher (5) shows that the production of carbon dioxid by surviving muscles becomes fairly constant after the second or third hour of survival. Therefore the uniform procedure was adopted of substituting the adrenalin solution as nearly as possible at about the end of the third hour of survival. The usual mode of experiment was as follows. A frog was decapitated, skinned, and the two hind legs cut off from the trunk at the pelvis. As a rule both hind legs were used. In each the muscles were cut free from their insertions at knee and ankle and separated as much as possible, without injury, by cutting the fascia which held them together. Both legs were then placed in *B* with 25 cc. of Ringer solution; the apparatus was closed and the machine run for a couple of hours; during this time no readings were made, as a rule, and the carbon dioxid was absorbed in the NaOH absorption chamber of the apparatus. At the end of this time the machine was opened and the muscles rinsed and covered with fresh Ringer solution. At the same time 25 cc. of freshly prepared adrenalin solution were put in *A*. The apparatus was closed, the pump started, and the rate of respiration determined for the next hour or, if necessary, until the rate of carbon dioxid production seemed to be fairly uniform. Then without stopping the pump, or in any way disturbing the apparatus, the three-way stop-cock, *Y*, was turned so as to drain the contents of chamber *B* into *C*. The cock was then returned to its former position (the one shown in the diagram) and, immediately, *X* was turned so as to drain the adrenalin solution from *A* into *B* and into contact with the muscles; this cock was then returned to its former position (as shown in the diagram). In the meantime, as has been said, the machine was running undisturbed, and it was possible to begin making a reading immediately after letting the adrenalin solution onto the muscles. These manipulations require less than a minute and were usually made after forcing the indicator to the alkaline standard, pH 7.36; the time required for it to change to the acid standard, pH 7.09, could then be determined at once and the reading was usually completed within about five minutes after adding the adrenalin solution. In other words, this procedure makes it possible to determine the immediate effect of the solution to be tested because, in the first place, the closed system need not be disturbed by opening it, and, in the second place, during the

preliminary hour or so, the two solutions—the pure Ringer that the tissue is in and the Ringer containing adrenalin—have had time to come into equilibrium with such carbon dioxid tensions as exist within the closed system and any disturbances due to inequalities of that nature are ruled out.

Careful control experiments with Ringer solution showed that the principle of the device is sound and that no disturbance is caused by the mere transfer of the solutions from one cup to another. In such cases the normal course of carbon dioxid production was not altered

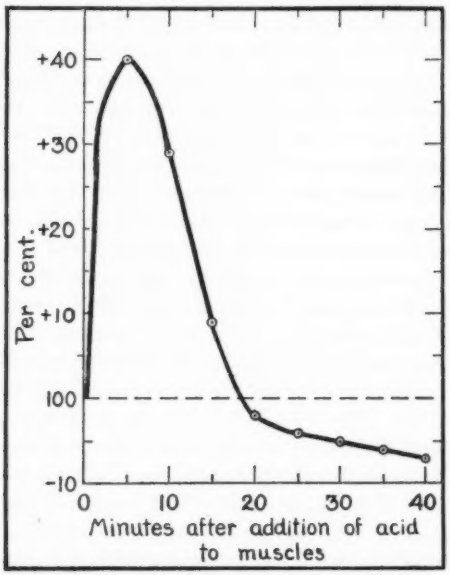

Fig. 2. This graph shows the average of ten experiments in which 1:50,000 adrenalin chloride solution (1. cc. of 1:1000 made up to 50 cc. with neutral Ringer) was added to the hind leg muscles of the frog.

The variations in rate of carbon dioxid production from the control period are expressed in percentage.

The graph is purposely labelled as showing the effect of *acid* upon the carbon dioxid production of muscles, because, as explained in the text, the adrenalin chloride solution was found to have the effect which is shown here, by virtue of its acidity.

The main purpose of this figure is to show the competence of the technique employed to detect *immediate* variation in the carbon dioxid production of the tissues.

in any way. On the other hand, figure 2 shows that the method is quite competent to detect immediate variations in the rate of carbon dioxid production. Figure 2 gives the average of ten experiments in which the equivalent of an approximately N/50,000 HCl solution was added to the muscles at the beginning of their fourth hour of survival. Further details of these experiments will be given later, but they are mentioned here to illustrate the competence of this method to detect any immediate effects upon the rate of carbon dioxid production of tissues. It is thought that this is probably a more trustworthy technique than that employed by Hutchinson.

1. Experiments with adrenalin chloride. This was used in a concentration of 1:50,000, which was made up by diluting 1 cc. of the 1:1000 solution to 50 cc. with Ringer solution. Such an adrenalin chloride solution has an acidity of between pH 4.4 and 4.6. In other words, the solution is as acid as an approximately N/50,000 HCl solution. A number of experiments made with such solutions of adrenalin chloride, before their acidity was recognized, gave the results shown in figure 2. Not much imagination is required, however, to see that such effects might be interpreted as due to decomposition of the carbonates and bicarbonates within the tissues by the action of a relatively acid solution. And experiments in which, instead of adrenalin chloride, equally acid Ringer solutions were used, confirm this assumption. For this purpose, enough HCl was added to the neutral Ringer solution to bring it to the same tint (using methyl red) as the 1:50,000 adrenalin chloride solutions. These acidified Ringer solutions produced just such an increase in the carbon dioxid production as is shown in figure 2.

Differences in hydrogen ion concentration between the Ringer solution used for control and the adrenalin chloride solution to be tested had, therefore, to be eliminated before the effect of adrenalin alone could be determined. It did not seem expedient to do this by using equally acid Ringer solution for the control periods because of the extremely unphysiological character of an acidity in the neighborhood of pH 4.4. Neither was it desirable to employ neutral or alkaline solutions in this work because of the well-known fact that adrenalin decomposes in such solutions. Tests showed that pH 6.6 to 6.8 was the lowest hydrogen ion concentration at which the adrenalin was sure to keep for such time as was required of it for these experiments. It was, therefore, decided to work at the lower of these values.

Two ways suggested themselves of obtaining this hydrogen ion concentration in both the control Ringer and the adrenalin solutions.

One was to add enough NaOH to the adrenalin solution to bring it to a pH of 6.6 and enough dilute HCl to the Ringer solution to bring it to the same pH value. Trial of this method showed that it was extremely difficult to control the hydrogen ion concentration by using pure acid and alkali. Recourse was had, therefore, to a much easier method. Enough M/5 Na_2HPO_4 was added to the acid adrenalin solution to produce the required pH of 6.6. Then, in order that the adrenalin and Ringer solution should have the same buffer action, and also to eliminate any effect of inequalities in the concentration of the phosphate ion, the same amount of Na_2HPO_4 was added to the Ringer solution, used for control. Since the Ringer was neutral to start with, the addition of this di-basic phosphate made it slightly alkaline; it was therefore brought back to pH 6.6 by titration with dilute HCl. It was found very easy to secure identical pH values of the two solutions in this way. Their buffer action was tested by titration to a definite end point with dilute HCl and also by determining their acidity (with indicators) when in equilibrium with the same tensions of CO_2, i.e., in equilibrium with room and alveolar air. These tests showed no appreciable difference in this respect between the two solutions when made up in this way.

Such adrenalin chloride solutions of 1:50,000 dilution and controlled for pH and buffer effect as described above, and used with the apparatus and according to the technique previously described, had absolutely no effect on the rate of carbon dioxid production of the skeletal muscles of the frog.

2. Experiments with adrenalin powder. Since adrenalin is a base, solutions of the powder are alkaline, so that 1 mgm. of it dissolved in 50 cc. of neutral Ringer produced an alkalinity of pH 7.4±; and the same amount in 25 cc. was proportionally more alkaline, having a pH in the neighborhood of 8. Again it was decided to work at pH 6.6 because of the better keeping qualities of the adrenalin at that acidity. Therefore enough M/5 NaH_2PO_4 was added to the Ringer solution to give it a pH of 6.6. The same amount of NaH_2PO_4 was then added to the alkaline adrenalin solution, in order to equalize the buffer effect and the phosphate concentration of the two solutions. This amount of di-acid phosphate was not enough to bring the alkaline adrenalin solution completely to pH 6.6, so it was brought the rest of the way by titration with dilute HCl. In both cases the use of the phosphates is justified, because of the greater ease and certainty with which a given pH value can be obtained and maintained by their means. And by

having them present in equal and minute amounts,[2] in both the control Ringer and the adrenalin solution, it is not thought that any appreciable error can be introduced either by virtue of their effect as phosphate ion, or by producing differences in buffer action. In fact, as has been said, this latter was checked, in both cases, and found to be the same.

When these precautions were taken to have the adrenalin solution and Ringer solution of the same pH and of the same buffer action, and using the modified Osterhout technique previously described, absolutely no effect was obtained on the rate of carbon dioxid production of the muscles with adrenalin powder in concentrations of 1:50,000, 1:25,000 and 1:10,000.

As a conclusion to this discussion of the effects of adrenalin on carbon dioxid production, it may be worth while to review briefly the observations reported by Hutchinson. She worked with solutions of adrenalin powder and, it will be recalled, found that all the concentrations which she used caused a depression in the rate of carbon dioxid production. With 1:33,333 and 1:50,000 solutions, this initial depression amounted to as much as 85 per cent and the rate remained permanently below normal. With concentrations of 1:200,000, 1:500,000 and 1:1,000,000 the initial depression was in the neighborhood of 30 per cent; this was followed by peculiar rhythmical variations and, finally, a return of the rate to normal within 45 to 60 minutes.

As a consequence of the negative results which I obtained with solutions of adrenalin powder, when controlled for pH value and buffer effect, it might be thought that Hutchinson's results were due either to the buffer action, or the specific effect of the alkalinity of the solutions. She states that the solutions of adrenalin which she used had no appreciable buffer action. That would leave her results to be explained as due entirely to the specific action of the alkali on the muscles. It seemed worth while to test this point. The means finally adopted for

[2] It required slightly less than 0.15 cc. of an approximately M/5 Na_2HPO_4 solution to produce the required reaction in 100 cc. of the 1:50,000 adrenalin chloride solutions. This amounts to only 0.0106 gram per 100 cc. But since this is just about double the amount of phosphate in Tyrode's solution it may be regarded with some suspicion. The required reaction was obtained in the solutions of adrenalin powder with 0.05 cc. of an approximately M/5 NaH_2PO_4 solution in 100 cc. This amounts to something like 0.0019 gram per 100 cc. and is much less than the amount of phosphate in Tyrode's solution. It is, therefore, improbable that its use could be physiologically objectionable. For this reason most of the work was done with the adrenalin powder.

this purpose are shown in figure 1 B. The only features of this apparatus which are not self-explanatory are the parts *X* and *Y*. These are identical in construction and each consists of a piece of glass tubing which projects through the stopper and is provided with a tightly fitting, solid glass rod for a plunger. A piece of rubber tubing slips over the outer end of the glass tube and grasps the plunger so as to make an air-tight junction, but one which, at the same time, permits free motion of the plunger. Before beginning an experiment the stoppers are inverted; a small piece of cotton is tamped into a small, compact wad against the inner end of each plunger; on each wad of cotton is placed a weighed amount of adrenalin powder; finally, a paraffined cork is used to stopper the end of the glass tube and the plunger is pushed in so as to bring the cotton wad and the adrenalin powder against the cork.

Hutchinson obtained maximal depressions of the rate of carbon dioxid production with 1:50,000 and 1:33,333 (0.002 and 0.003 per cent) concentrations of adrenalin. I therefore decided to work with 1:35,000 solutions. Both *X* and *Y* were accordingly loaded with 1 mgm. of adrenalin powder; 35 cc. of Ringer were put in both *A* and *B*, and also, both hind legs of a frog were placed in *A*. The stoppers were inserted in *A* and *B*, the pump was started and the rate of carbon dioxid production determined for an hour or more. Adrenalin could then be added to either solution without stopping the pump, opening the machine, or in any other way disturbing the equilibrium within the system, by simply pushing the proper piston a very short distance.

Figure 3 shows graphically the result of a single experiment in which adrenalin was added, first to the muscles and secondly to the Ringer in chamber *B*. Four other experiments showed equally well that it made no difference whether the adrenalin was added to the muscles or to the Ringer in *B*. There was no uniform difference nor any which was not easily within the limit of error involved in making the color determination, or in weighing out the adrenalin. Since adrenalin in *B* can have an effect only by virtue of its buffer action, this would seem to be the principal thing that Hutchinson must have measured in her experiments. My experiments showed depressions of from 15 per cent to 25 per cent in the rate of carbon dioxid production with a prompt return to normal. So they fail to account for the very great depressions observed by Hutchinson, with even less concentrated solutions. But whatever her results may have been due to, I believe the evidence presented here shows that they very probably were not due to any specific action of adrenalin itself.

II. The effect of adrenalin upon total acid production. The work of Martin and Armitstead still remains to be considered. In spite of the apparent discrepancy between my results and those which were reported by them, there is no reason for doubting either, because the two lines of work are not strictly comparable. What was measured in my experiments was carbon dioxid production, and nothing more, whereas they measured the total acid production of their muscles. From what we know of normal metabolism there would be no *a priori* reason for doubting that any increase in total acid production could be largely accounted for by increased carbon dioxid production. But the conditions of all these experiments were anything but normal and the conditions of my experiments were far more abnormal than theirs.

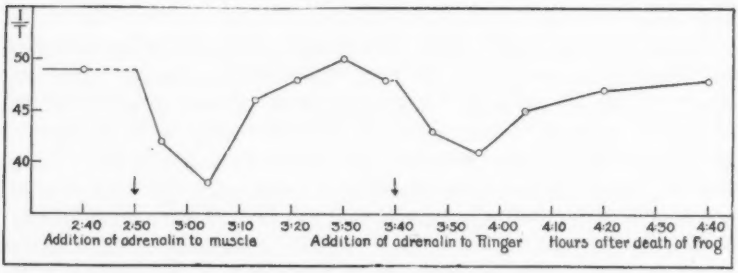

Fig. 3. This shows graphically the result obtained in a single experiment with the use of adrenalin powder and the device shown in figure 1B.

The rates of carbon dioxid production are obtained by taking the reciprocal of the time required for the indicator to change from pH 7.60 to pH 7.36.

In the first place I was working at pH values which were decidedly acid (pH 6.6) whereas normal tissue reactions are slightly alkaline; and what is more significant, adrenalin is fairly stable at the hydrogen ion concentration which I used, whereas it is less stable and, it might be assumed, physiologically more active at such hydrogen ion concentrations as exist within normal tissues. Martin and Armitstead presumably worked on the alkaline side of neutrality because the indicator they used is only serviceable between pH 7 and pH 8, and they therefore have the benefit of any doubt that might exist as to this point. Theoretically, I do not believe much weight is due to this consideration because such adrenalin solutions as I used and even more acid solutions produced the usual inhibition of intestinal strips and frog stomachs; furthermore, my muscles seemed to retain their excitability and con-

tractility to a high degree in such solutions. Nevertheless, tissue respiration may be more sensitive to hydrogen ion concentrations than the processes involved in contractility.

A more significant difference between their work and mine, however, lay in the fact that they tested their muscles just as soon as they could be dissected out of the frog, whereas my technique involved waiting until the normal carbon dioxid production had reached a fairly uniform level, i.e., until about four hours after death. For these reasons, it was decided to repeat their work as nearly as could be determined from their description of it and using, as they did, solutions of adrenalin powder.

The experiments were begun with 1:25,000 solutions which, according to them, should give about maximal results. Such a solution, made by dissolving 1 mgm. of the powder in 25 cc. of Ringer solution, has a pH, as mentioned above, of about 8. Since it was desired to have the hydrogen ion concentration as nearly as possible like that normal for tissues, it was decided to work between the end points pH 7.36 and pH 7.09. Enough dilute HCl was therefore added to the adrenalin solution to bring it to pH 7.36. Martin and Armitstead say they added bicarbonate to their Ringer solution so I added enough sodium bicarbonate to my neutral Ringer solution to give it a pH of 7.36. A sartorius muscle, recently dissected out, was placed in each solution and, as they reported, the adrenalin solution showed an increase in acidity, to the chosen end-point of pH 7.09, much more rapidly than the pure Ringer solution. About this time, however, the use of sodium bicarbonate began to arouse suspicion. This salt is one of the most efficient buffers we have and the addition of it to one solution and not to the other seemed to establish an undesirable difference between the two. To be sure, the adrenalin powder is alkaline; but it might have an altogether different buffer action from the bicarbonate.

What was done, therefore, was to neutralize the alkaline adrenalin solution with dilute HCl. Enough sodium bicarbonate solution was then added to it to bring it to approximately pH 7.36. Exactly the same amount of bicarbonate was added to the neutral Ringer solution. Any difference in pH of the two solutions was then adjusted with dilute HCl, so that they both had the same pH and were approximately at 7.36. Now, when one member of a pair of sartorius muscles was placed in each solution the color changes of the solutions and their arrival at the end-point of pH 7.09 were so nearly parallel and simultaneous that no significant difference could be detected between them. The buffer

action of these solutions, as thus made up, was tested by means of titration and also by their acidity when in equilibrium with definite concentrations of carbon dioxid—e.g., room and alveolar air—and found to be practically the same.

Adrenalin powder, in strengths of 1:50,000, 1:25,000 and 1:10,000, was tested in this way and found to have no effect on the rate of acid production.

It would seem to follow, therefore, that the results of Martin and Armitstead may have been due to the fact that they failed to control properly the buffer action of their solutions. The fact that they observed increases in acid production which were more or less proportional to the concentration of the "adrenalin" solution, with concentrations up to about 1:25,000, but no further increase with concentrations above this, can probably be explained as follows. The alkalinity of the solutions of adrenalin powder will be proportional to their concentration and, consequently, if any attempt were made to make the Ringer solutions even approximately as alkaline—with bicarbonate—as the adrenalin solutions, the buffer action of the Ringer solution would vary in proportion to the adrenalin concentration of the adrenalin solutions. Therefore, the more concentrated the latter, the better buffered would be the Ringer against any increase in acidity. On the other hand, an adrenalin-powder solution of 1:25,000 concentration has an alkalinity in the neighborhood of pH 8 and this is the limit of usefulness of the indicator they used. It is as pink at pH 8 as it will become. Consequently the increased alkalinity of more concentrated adrenalin solutions would not be observed and, more particularly, it would furnish no inducement to increase the alkalinity of the Ringer solutions—or, better, their buffer effect—in proportion to the adrenalin concentrations. And, since the buffer action of the alkaline adrenalin powder seems small in comparison with that of anything like equally alkaline bicarbonate solutions, the proportionality between the adrenalin concentrations and the *apparent* rate of acid production would cease to exist. The description which they give of their technique is not so full as one would like to have before making such a criticism; the only point I wish to emphasize, therefore, is, that when their technique is followed as nearly as their description permits, but precaution is taken to equalize the buffer action of the solutions used, no effect of adrenalin on the acid production of isolated muscles can be detected.

One question of importance has not been considered as yet and that is whether or not the failure to obtain any effect upon the respiration

of the muscles might be due to destruction of the adrenalin before it has had time to act. Reference has been made to the fact that it spontaneously decomposes in neutral or alkaline solutions, and Hutchinson explained the prompt return of the carbon dioxid production to normal, on the assumption that the adrenalin might have lost its strength. One is therefore anxious to know how far this goes within the time of these experiments and also whether some substance in or given off by the muscles might hasten this decomposition, even in the acid solutions that were used. It is felt that figures 4 and 5 show clearly

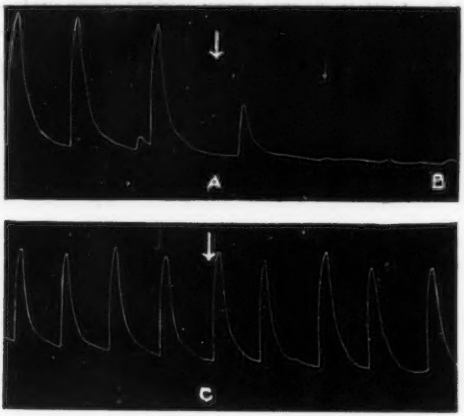

Fig. 4. The record is that of a beating frog stomach immersed in 25 cc. of Ringer solution at room temperature (21.5°C.).

Twenty-five cubic centimeters of Ringer solution and 25 cc. of 1:25,000 solution of adrenalin powder were both brought to a pH of 7.36, as described in the text. In each was placed one of the hind legs of a frog. Two hours later, 1 cc. of the adrenalin solution, added to the 25 cc. of Ringer solution bathing the stomach, inhibited the stomach contractions, as shown at *A*. At *B* the stomach was put in fresh Ringer where it resumed its beating. Ten minutes later, at *C*, 1 cc. of the Ringer "extract" of frog leg was added to the fluid bathing the stomach. The record shows there was no inhibition, so the inhibition observed at *A* must have been due to the adrenalin in the solution and not to any materials merely extracted from the muscles during their immersion in the fluids.

A simple calculation shows that the amount of adrenalin in the solution bathing the stomach at *A* could not exceed 1:625,000 and would be less than that in proportion to the amount which was decomposed during the period in contact with the muscles. Since Biedl states that frog stomach is not sensitive to concentrations of adrenalin less than 1:1,000,000 it would seem improbable that much of the adrenalin was decomposed under the conditions of these experiments.

that even in alkaline solutions the concentration of the adrenalin cannot have been very seriously diminished during even the longest periods used in any of these experiments.

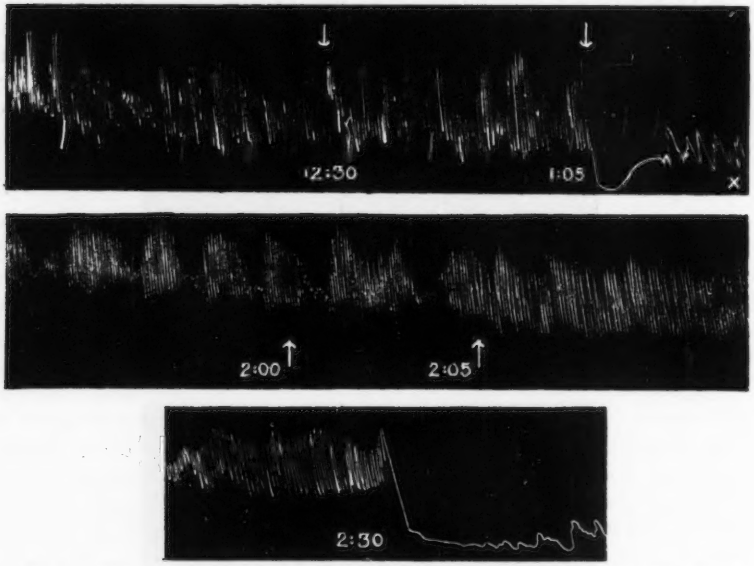

Fig. 5. Solution "A:" At 11 a.m. the skinned hind legs of a frog were put in 25 cc. of Ringer solution, made up to a pH of 6.6 as described in the text.

Solution "B:" At the same time (11 a.m.) the skinned hind legs of another frog were put in the respiration chamber of the machine with 25 cc. of 1:50,000 adrenalin chloride solution of pH 6.6 and the pump started.

The record is that of a beating strip of rabbit intestine in 100 cc. of warm (37°C.) oxygenated, mammalian Ringer solution.

At 12:30, 5 cc. of solution "A" were added to the solution in which the intestinal strip was beating. Mechanical disturbances due to striking the writing lever with the pipette somewhat obscure the result, so the same thing was done at 2:00 and 2:05. These three trials show that this amount of such a muscle "extract" has no effect on the activity of the intestinal strip, or if there is any effect it is to increase the contractions.

In contrast, is the effect of solution "B." At 1:05 and also at 2:30, 5 cc. amounts of this solution markedly inhibited the activity of the intestinal strip when added to the mammalian Ringer in which it was immersed. This would seem to indicate that adrenalin under the conditions and in the concentration used in these experiments, remains active even after three or more hours in contact with frog muscles.

New, warm, mammalian Ringer was substituted at *X*.

Before concluding, it must be pointed out that the failure of adrenalin to affect the carbon dioxid and total acid production of muscles, *under the conditions of these experiments*, does not permit any conclusions as to the relation of the *adrenal glands* to metabolism (6). That is a different problem. Neither do these results permit a criticism of experiments in which adrenalin chloride has been found to affect the metabolism of cats and man (7). It does not seem, however, that the strong acidity of the adrenalin chloride solutions was recognized in any of the experiments in which its metabolic effect has been tested, and the preceding experiments simply suggest that it might be wise to control this factor in future work.

SUMMARY

1. A modification of the Osterhout respiration apparatus is described, which makes it much more serviceable in detecting the immediate effect of substances in solution upon the carbon dioxid production of the organism or tissue that is being studied.

2. By means of this technique, and particularly by controlling such factors as the pH and the buffer action of the solutions used, it is shown that adrenalin has no effect upon the carbon dioxid production of surviving frog muscles. This applies to both the adrenalin chloride solution and adrenalin powder when used in dilutions from 1:10,000 to 1:50,000.

3. It is also shown that adrenalin has no effect upon the total acid production of isolated, skeletal muscles of the frog.

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ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

III. Immediate Changes in the Flexion Reflex after Spinal Transection

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Received for publication February 10, 1923

The effect of transecting the spinal cord upon reflexes involving centers below the plane of transection has been discussed by several authors. In the older literature (1), (2), (3), (4) it was stated that the immediate effects of spinal transection in all vertebrates were profound depression of all the reflexes involving these lower centers, the depression being greatest in man, next greatest in the monkey, and less marked in the cat and dog. This view has been modified, however, at least as regards the nervous system of the cat, by the observation of Sherrington and Sowton (5) that in this animal the flexion reflex of the hind limb as evoked by single induction shocks, shows an immediate increase following transection, instead of the depression which had hitherto been supposed to be general. They found the threshold of reflex excitation actually lowered by spinal transection in the case of this reflex, and that the response to a stimulus of given strength was increased in magnitude. In a recent paper by one of us (6, p. 383) it has been pointed out that the after-discharge in the case of the flexion reflex had been mentioned by Sherrington as being specially subject to depression by spinal transection, and that since in the earlier days it was customary to use repeated stimuli to evoke all reflexes, the disappearance of the after-discharge and with it of the cumulative effect of a rapid series of stimuli, would readily explain the earlier impression that the flexion reflex partakes in the general depression of the reflexes of the lower centers. Further evidence on this point will be discussed later in the present paper. It has seemed to us worth while to examine the immediate effects of spinal transection on the flexion reflex with the method of observation described in the first paper of this series (7), namely, the recording of action currents in a motor nerve involved in

the reflex. The flexion reflex is a useful one for study since it can be evoked by a single stimulus applied to an afferent nerve. The reflex arc, thus excited and its responses recorded by leading off from a motor nerve, constitutes perhaps the simplest reflex preparation that may be used, for the receptors, the nerve-muscle junction and the muscle fibers are eliminated, and with them the possibility of sources of confusion, the preparation consisting only of nerve fibers and synapses, and this reduced to the simplest possible terms.

METHOD. Our experiments were performed in two distinct groups, the first group in the summer of 1916, the second group in the spring of 1921. Nearly all the essential details of technique and apparatus have been described in the first paper of this series (7). The essential points of our standard procedure are as follows: The animal (cat) was decerebrated under deep ether anesthesia, the transection being always approximately at the level of the anterior colliculi. The sciatic nerve was exposed in the thigh and its two main branches, peroneal and popliteal, separated by careful dissection as high as the hip. Sherrington shielded electrodes (8) for stimulation were applied to the popliteal nerve severed in the popliteal space. The peroneal branch was severed at the point where it enters the *tibialis anticus* muscle, crushed at a point about 1 cm. from the cut end in order to render the action currents monophasic, and led into a moist chamber where it was placed on a pair of non-polarizable "boot" electrodes with the crushed point between them. Thus far the procedure is essentially that which has been described (7). In these experiments, besides the above procedure, the spinal cord was exposed for 1 or 2 cm. at the level of the last rib, the wound being closed over it until we should be ready to make the transection. This preliminary operation was performed after decerebration, and before the preparation of the branches of the sciatic nerve.

For stimuli we regularly used single break shocks from a Berne inductorium calibrated in accordance with Martin's scale (9). The recording apparatus used in the first group of experiments in 1916 was precisely the same as that described in the first paper (7), consisting of a Cambridge string galvanometer and the film camera described in that paper. In the second group of experiments in 1921 we used a Hindle string galvanometer and the recording camera described in a more recent communication (10, p. 418). In some of these latter experiments we also made use of the electron-tube method of amplification as previously described (10).

Since in the first paper of this series some evidence appeared suggesting a gradual progressive increase in the size of response in the flexion reflex after decerebration (7, p. 173), it seemed to us important to establish a good base line in order that any increase in the size of response following transection should not be confounded with such a progressive change. To determine the extent of such a possible source of error we performed five control experiments in which records of the flexion reflex were made at intervals over the entire range of time normally occupied by a complete experiment, but without spinal transection. In certain experiments in order to determine to what extent such progressive increase in the size of response as might occur depended on the time after decerebration, and to what extent it was due to changes which might result directly from setting up the nerve in a moist chamber we attempted to make records alternately from corresponding reflex preparations in the right and left leg. In doing this, however, we encountered a difficulty in that the nerve which had been used on the side first exposed, lying between the dissected tissues in the closed wound while the dissection was being made on the other side, ceased to conduct, and thus rendered it impossible to repeat the observations on the side first exposed (11). We therefore found that the best way of obtaining a good base line was to leave the preparation undisturbed with the motor nerve in the moist chamber for a considerable time, varying from 15 minutes to 3 hours, before transection was made. Having by repeated observation determined the magnitude of response to induction shocks of various strengths, including both maximal and submaximal, we proceeded to transect the spinal cord as rapidly as possible. In the earlier experiments the motor nerve was removed from the moist chamber during transection, and then replaced with the electrode contacts as nearly as possible the same as before. In the later experiments, in order that there should be no shift of contact of the motor nerve on the electrodes, the animal was held securely by an assistant while the transection was being made. As soon as disturbing muscular contractions had ceased responses to the same stimuli as before were recorded. This was first done at a time varying from $1\frac{1}{2}$ to 15 minutes after the moment of transection.

Results: *Control experiments.* In a majority of the control experiments the galvanometric excursions increased slightly during the first hour or so after the motor nerve was set up in the moist chamber. The evidence was not uniform in demonstrating any clear increase in response correlated with the time after decerebration; furthermore,

whatever increase this or previous evidence (7) indicates is at most very gradual. The distinct increase usually found to follow the setting up of the nerve in the moist chamber is therefore probably to be ascribed either to the drying of the excess of moisture which provides a partial short-circuit for the action current, or to the recovery of the nerve from damage which it may have sustained in handling, and which may have caused it to conduct with a decrement (11). At all events, the change occurring while the preparation remained undisturbed with the nerve on the electrodes was so small and so gradual that it was not likely to be a source of confusion or error. Furthermore, each experiment included its own control, in that before transection responses were recorded with the preparation otherwise undisturbed over a long enough time to establish a satisfactory base line for comparison with the responses after transection.

Single stimuli. In the first paper of this series it was shown that the size of reflex response is greatly influenced by the period of rest allowed to elapse after a previous stimulus before testing. If a rest of 5 or 10 seconds is allowed before each stimulus the size of the responses will be fairly uniform. If a series of stimuli is applied with a frequency of 4 or 5 a second, for example, there is a striking decrease in the size of response after the first (fig. 3). In order to make a valid comparison of results before and after transection we recorded a considerable series of responses to break shocks of constant strength, carefully allowing at least 5 seconds to elapse before each stimulus. Our measurements were made mostly on break shocks; in a few cases they were supplemented by measurements on make shocks.

Fourteen experiments were performed in which good records were obtained. Of these, twelve showed a definite increase in the size of response after transecting the cord. The only two exceptions which appeared to show the opposite result were animals which became moribund and died so soon after transection that the results may fairly be excluded from the series. Typical records showing responses to stimuli of equal strength immediately before and after transection are shown in figure 1. It is noteworthy that in figure 1 *B* the response shown after transection was recorded only a minute and a half after the cord was transected. Table 1 shows the averages of measurements of excursions of the string as measured on the film with stimuli of various strengths, from a series of typical experiments. The figures designated *A* are from records with electron-tube amplification. By comparing the magnitudes of the excursions it can be seen that most

of the stimuli are evidently maximal for the afferent nerve (cf. 12, 13). Figure 2 shows a series of curves, each from a single experiment, in which the size of response to a stimulus of constant strength is plotted against time, and the immediate increase in size of response following transection is clearly shown.

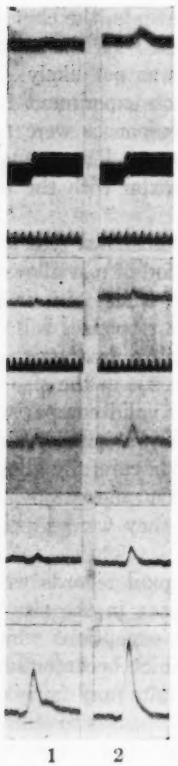


Fig. 1. Monophasic reflex responses before and after spinal transection in five different experiments. *1*, before transection; *2*, after transection. In *A* and *B* time is shown by tuning fork shadow at bottom, 1 double vibration = 0.01 second. In *A* the time of stimulus is shown by rise in middle line; upper line is galvanometer record. In *C*, *D* and *E*, electron-tube amplification was used with tight string and 0.1 microfarad condenser damping (Einthoven, 21); electrical artefact causing preliminary displacement of shadow shows time of stimulus.

A, June 2, 1916; *1*, 2 minutes before transection; *2*, 8 minutes after transection. Stimulus in both 405 Z. 5000-ohm platinum string; tension, 215 m. per amp. (see Forbes and Ray, 11). Magnification, 580.

B, April 14, 1921; *1*, 1½ minutes before; *2*, 1½ minutes after. Stimulus 113 Z. 25,000-ohm gilded quartz string; tension 280 m. per amp. Magnification, 490.

C, April 5, 1921; *1*, 8 minutes before; *2*, 21 minutes after. Stimulus 167 Z. 25,000-ohm string; tension, 51 m. per amp. Magnification, 490. Amplification approximately 20-fold. Resistance of nerve, 50,000 ohms. Speed of film, 14 cm. per sec.

D, May 17, 1921; *1*, 11 minutes before; *2*, 3 minutes after. Stimulus 111 Z. 25,000-ohm string; tension 102 m. per amp. Magnification, 490. Speed of film, 18.5 cm. per sec.

E, May 19, 1921; *1*, ½ minute before; *2*, 3 minutes after. Stimulus 113 Z. 25,000-ohm string; tension, 102 m. per amp. Magnification, 490. Speed of film, 18.5 cm. per sec.

In three of the experiments we made measurements of the reflex time before and after transection, measuring several records in each experiment. In the earlier experiments this was done by means of a signal magnet which cast its shadow on the film; in the experiments in which electron-tube amplification was used, the artefact due to the stimulating current revealed the time of stimulation (14, p. 130). These

TABLE 1

DATE OF EXPERIMENT	TIME OF TRANSECTION AFTER DECEREBRATION	STRENGTH OF STIMULUS Z UNITS	AVERAGE EXCURSION	
			Before transection	After transection
			mm.	*mm.*
June 2, 1916	2 hr. 28 min.	199	0.5	1.2
		285	0.5	1.5
		405	0.2	1.3
June 12, 1916	2 hr. 20 min.	14	1.1	1.1
		18	1.3	1.3
		22	1.0	1.1
		30	1.0	1.3
		41	1.1	1.2
August 2, 1916	2 hr.	8	1.2	1.3
		16	1.3	1.4
		28	1.1	1.3
		67	1.1	1.3
		264	1.4	1.5
March 24, 1921	6 hr.	79	0.5	0.9
		113	0.7	1.0
		160	0.8	1.0
		199	0.9	1.0
April 5, 1921	6 hr. 8 min.	167	1.2*A*	3.1*A*
		167	0.2	0.4
		207	0.1?	0.5
April 14, 1921	5 hr. 52 min.	79	0.6	0.8
		113	0.3	0.9
May 17, 1921	3 hr. 17 min.	81	1.0*A*	2.1*A*
		111	0.9*A*	2.4*A*
		158	0.7*A*	2.2*A*
May 19, 1921	4 hr. 20 min.	58	5.3*A*	11.0*A*
		79	5.4*A*	12.4*A*
		113	5.7*A*	11.6*A*
May 24, 1921	3 hr. 8 min.	47	1.0	1.0
		60	0.9	1.0
		82	0.9	1.1
		118	1.0	1.0

measurements showed that following transection there was no appreciable change in the latency.

The effect of a rapid series of stimuli. The effect of spinal transection in increasing the size of responses to isolated stimuli is striking, but even more striking is the effect on response to a rapid series of stimuli such as produces, in the decerebrate preparation before spinal transection, a rapid decrease in the size of response, suggestive of fatigue. After spinal transection this diminution on repetition almost disap-

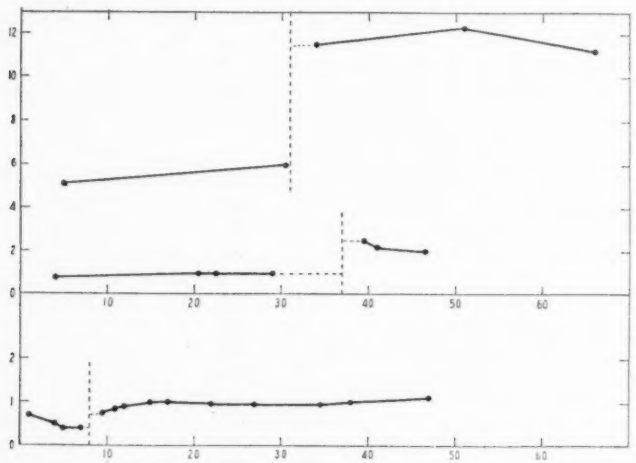


Fig. 2. Curves showing galvanometric excursions recording monophasic responses to maximal stimuli (constant strength) plotted against time in three experiments. The time of spinal transection in each experiment is shown by the broken line. The two upper curves are from responses amplified by the electron tube; the lowest curve is from records with unaided galvanometer. Abscissae, time in minutes; ordinates, excursions in mm. Top curve, May 19, 1921; middle curve May 17, 1921; lowest curve, April 14, 1921.

pears. In some of our experiments we were unable to detect any falling off in the size of response, all those in a series being as large as the first, as nearly as we could measure them. Other preparations, however, with larger responses which could be more accurately measured, showed that after transection there still was a slight diminution in size of response after the first of a series. Typical examples of this are shown in figure 3. Corroborative evidence was found in a considerable series of measurements of the electric response of the *tibialis anticus* muscle and

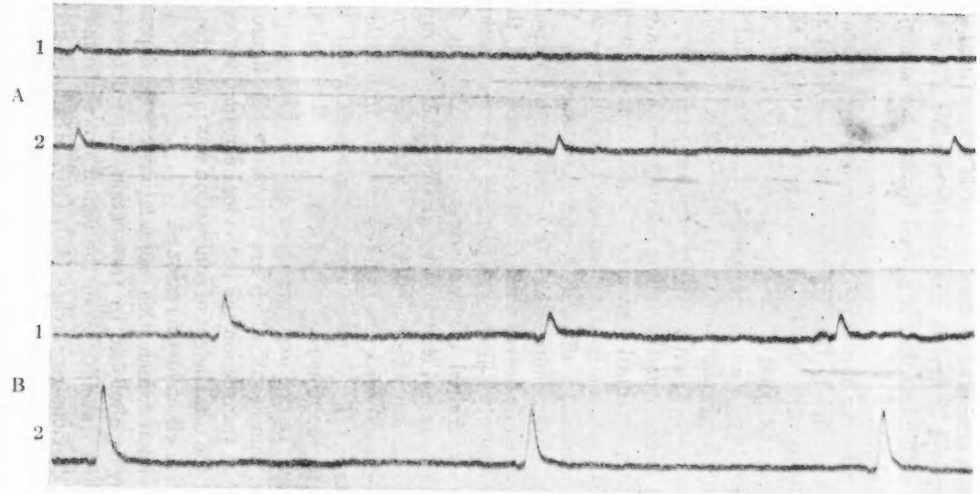


Fig. 3. Effect of spinal transection on responses to a series of stimuli, shown in two experiments, *A* and *B*. In each experiment, *1* is before transection: *2*, after. A rest of several seconds preceded the first stimulus of each series. Responses to break shocks only; make shocks subminimal. Electron-tube amplification in both experiments.

A, May 17, 1921; *1*, 14 minutes before transection; *2*, between 3 and 4 minutes after. Stimuli 111 Z.

B, May 19, 1921; *1*, less than 1 minute before transection; *2*, 3 minutes after. Stimuli, 113 Z.

In both experiments 25,000-ohm string; tension 102 m. per amp. Magnification, 490. Speed of film 18.5 cm. per second (as in fig. 1, *D* and *E*).

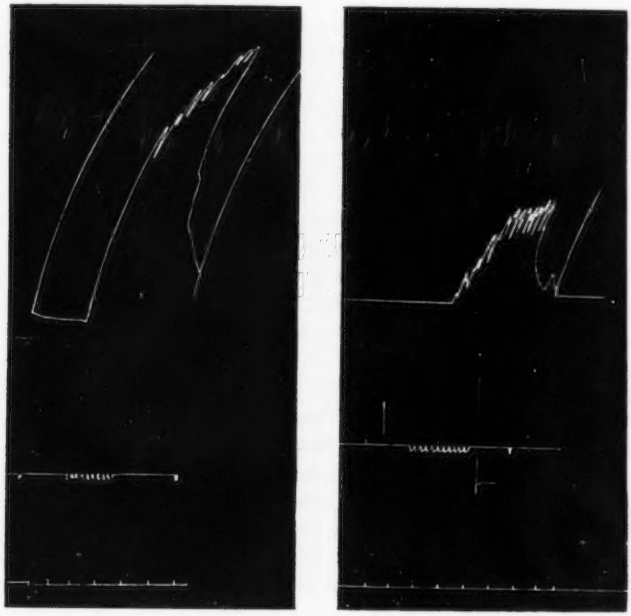
its mechanical contraction made in the course of some experiments on the flexion reflex already reported by Adrian and Forbes (13). These showed that with various strengths of stimulus, both submaximal and maximal, the reflex response in muscle was slightly smaller if the test stimulus was preceded within less than a second by another stimulus, e.g., an effective make shock.

The first impression this diminution of response conveys is one of synaptic fatigue. As has already been pointed out (7, p. 171) this view presents the difficulty that the fatigue is extremely rapid to a certain point, but beyond this point it does not seem to go; that is, the size of response falls rapidly to an approximately fixed level and remains there. This would imply that some part of the reflex mechanism fatigues rapidly and some other part is highly resistant to fatigue.

Another explanation is suggested by effect of transection on after-discharge as observed in mechanical responses. We have noted in the introduction that the after-discharge was mentioned long ago as that feature of the flexion reflex most affected by spinal transection. We noticed in watching one of our preparations in which the hip muscles were still in action, their motor nerves not having been sectioned, that a remarkable change in the after-discharge occurred after transection. Before transection the individual contractions were small, but the after-discharge was so pronounced that the cumulative effect of a rapid series of stimuli caused almost extreme flexion. After transection a similar series of stimuli caused brisk but isolated twitches with little or no visible after-discharge. They then more nearly resembled the responses of a muscle to motor-nerve stimulation than they did the reflex before transection. In one experiment we obtained myographic tracings of this change, samples of which are reproduced in figure 4. It will be seen that before transection the individual contractions were appreciably smaller, but the total height of contraction was much greater because of the cumulative effect just mentioned.

The after-discharge upon which this cumulative effect depends and which has been largely abolished by transection, must depend on a series of nerve impulses travelling from the center down the motor nerve to the muscle. Previous researches (15), (16), (17) have given some reason to believe that normally a motor-nerve fiber occupied in mediating a sustained muscular contraction (e.g., voluntary) conducts impulses at a frequency of several hundred a second; that is, each impulse arising before the end of the relative refractory phase following its predecessor. Under these conditions such a fiber would be refrac-

tory to further stimulation a large part of the time. It would alternate between an absolute refractory and a relative refractory state. In view of the all-or-nothing principle (18), (19), a fiber already engaged in conducting an impulse can do no more. If, then, an appreciable number of the fibers in a motor nerve under observation are engaged



A B

Fig. 4. Myographic records of flexion reflex in response to series of rapidly repeated stimuli.

A, 3 minutes before transection.

B, 5 minutes after transection. Synchronous ordinates before and after tracings, made with drum stationary. Upper line records contraction of *semitendinosus* muscle; middle line made by signal magnet shows make (fall) and break (rise) of primary current; lowest line shows time in seconds. Strength of break shocks, 82 Z.

in conducting a rapid series of impulses involved in the after-discharge, which is so clearly evident in the decerebrate condition before spinal transection, it is a necessary consequence that there will be fewer motor neurones available to respond to an afferent stimulus coming at this

time. The result of this would be a reduction in the electric response obtained from the nerve as a whole. The greater the after-discharge, the more fibers would be occupied and therefore refractory to further stimulation.

Even if the frequency of motor-nerve impulses is not as high as is suggested above, the same principle would apply. At a lower impulse frequency a given nerve fiber would be refractory a smaller percentage of the time, and hence the effect of after-discharge in reducing the size of the individual response would be somewhat less, but it would still be present. Thus the reduction in the size of response may not be a fatigue effect at all, but the result of increasing the number of fibers which are already busy and therefore incapable of further response.

One might expect to find in the galvanometer record during the interval between stimuli some evidence of such persistent activity in the motor neurones, but a consideration of certain properties of action currents in nerves will show that such a neural discharge as we have just pictured might well fail to reveal itself in the galvanometric record. The action current of a motor-nerve fiber is very brief; it is nearly over in a thousandth of a second (20). Even when all the fibers in the nerve are stimulated with a single shock and respond simultaneously the resulting deflection in the string galvanometer is not large. According to the proposed view of sustained neural discharge, each fiber involved is responding with a frequency such that each impulse, falling in the relative refractory phase following its predecessor, is subnormal. Therefore, if a small percentage of the fibers only are responding at all, and those not synchronously but each in a rapid series of subnormal responses, and all out of phase with each other, the result would be not a well-defined series of galvanometric excursions, but at most a small displacement of the base line. The galvanometer record reproduced in figure 3, and others obtained from the same preparation, show a slightly greater persistence of the electric response in the record before transection than in the corresponding record after it. The difference is appreciable for about 0.05 second after the beginning of each response; after that the irregularity of the base line in records such as this, made with electron-tube amplification, renders it impossible to tell whether there is a slight displacement or not. In those records made without electron-tube amplification it is impossible to detect any evidence of after-discharge for, although the base line here is steadier, even a maximal response only produces a small excursion of the string, and a displacement resulting from after-discharge, if it exists at all, must

be too small to be discernible. There is, however, in the slight persistence of the galvanometer excursion in the electron-tube record, disappearing after spinal transection, some direct evidence of after-discharge in the motor nerves, and of its partial abolition by transection. But even without this evidence we can hardly doubt that the after-discharge, so clearly shown in the mechanical record of the muscular contraction, must be set up by some sort of a stream of nerve impulses, and in view of the refractory phase which must be associated with these impulses, there is clearly a valid basis for the proposed explanation of the decrease in the size of the responses which occurs while after-discharge is in progress.

The myographic records show that although after-discharge is greatly reduced by spinal transection it is not altogether abolished. The persistence of a slight after-discharge after transection corresponds well with the persistence of a slight decrease in the size of response following the first of a series of rapidly repeated stimuli. An evenly sustained activity, such as after-discharge, appears at first sight difficult to explain in terms of nerve impulses as we know them in peripheral nerves where they have been shown to sweep rapidly along the conducting path, leaving it for a time refractory. Continued activity in such a tissue must be intermittent, not evenly sustained. But in a previous communication (6, p. 371) it has been argued that the sustained activity of the nerve centers may after all involve no other form of disturbance than the transient nerve impulse of the all-or-nothing type with its associated refractory phase. This interpretation of after-discharge depends on the fact that the spinal cord is known anatomically to contain an enormously complex system of branching paths through which nerve impulses arising from a common source might conceivably travel. The result of setting an elaborate network of this sort in action would be to establish an apparently continuous or sustained state of activity, even though in each individual conducting path the disturbance consisted of an intermittent series of transient nerve impulses. If the after-discharge depends on the opportunity for reverberation of nerve impulses in the great network of available paths in the central nervous system, it would not be surprising to find this persistent activity greatly reduced in amount by spinal transection which isolates the local centers from those extensive tracts leading to the hind-brain and cerebellum.

In this connection it is significant to note that Forbes and Miller (14) in recording the action currents of the ascending tracts of the brain

stem in the decerebrate animal on stimulation of the sciatic nerve with single break shocks, found that when the stimuli were similarly grouped into a rapid series delivered at four or five a second there was a similar falling off in the size of responses after the first of a series, presenting a picture extraordinarily like that found in the falling off of the individual responses in the flexion reflex before spinal transection. Applying the same interpretation to these observations we should infer that the ascending afferent tracts in the upper cord and brain stem become involved in a similar reverberation or after-discharge, following the first volley of afferent impulses. Acceptance of this view would support the conclusion that fiber tracts in the upper portions of the nervous system are involved in the reverberation which serves to sustain the flexion reflex in the decerebrate preparation without spinal transection.

This recalls the suggestion of Pike (4) that in the intact animal all spinal reflexes depend on long paths involving the brain, the local paths being incapable of conduction, that spinal shock consists in the interruption of the long paths by transection, and that recovery from it consists in the establishment of functional connection which enables the local paths to conduct. The fact that the flexion reflex suffers no interruption at all by spinal transection, and is indeed rendered immediately more vigorous thereby, shows Pike's suggestion to be superfluous as regards this reflex. Not only is the view that the local paths involved in this reflex after transection are normally functionless rendered unnecessary, but it seems to be actually disproved by the fact that the reflex time is no less after transection than before. It is hardly conceivable that the conduction time of a path extending from the lumbar enlargement of the cord to the brain and back again should be the same as that of a path lying wholly within the limits of the lumbar enlargement. If further proof were necessary, it should suffice to cite the measurements of conduction times already reported in previous papers. Forbes and Miller (14) found that when the sciatic nerve is stimulated at the same point and in the same manner as was its popliteal branch in these experiments, the first nerve impulses arrived at the medulla oblongata approximately 8σ after the stimulus was applied. Forbes and Gregg (7), working on decerebrate cats without spinal transection, with a technique identical with that used in these experiments, found that the impulses in the motor nerve reached the leading-off electrode approximately 8σ after the stimulus. Of that time more than 2σ must have been required for the motor-nerve impulses to reach the electrodes after leaving the cord. Therefore it is clear that in the

decerebrate preparation the motor-nerve impulses involved in the flexion reflex have actually left the spinal cord before any ascending impulses have reached the medulla oblongata.

We are speaking now only of the flexion reflex. The possibility that Pike's suggestion does apply to the crossed extension reflex, which is depressed by spinal transection, is another question.

Although there is no reason to suppose that the local paths of the flexion reflex conduct only after spinal transection, there is one feature of Pike's view which probably does apply to this reflex, viz., the participation of the long paths in the after-discharge. If we consider the total reflex effect of a series of stimuli, spinal transection does cause a reduction, and apparently by virtue of the interruption of long paths leading to the brain.

Some of the above considerations suggest a possible explanation for the increase in size of the reflex response to a single stimulus after spinal transection. Conceivably in the decerebrate animal the higher centers (cerebellum, hind-brain, etc.) give rise to a slight degree of tonus in the flexor muscles, i.e., to streams of nerve impulses in some of the motor neurones. Those motor neurones which are thus occupied would be unable to respond to the afferent impulses evoking the flexion reflex. Withdrawal of this source of activity by spinal transection would then enable the neurones thus made idle to respond to the afferent impulses and would increase the total response appearing under the conditions of our experiments.

SUMMARY

1. When the reflex arc of the flexion reflex is reduced to its simplest elements, the stimulus being applied to an afferent nerve and the response observed by recording the action current of a motor nerve, low spinal transection causes an immediate increase in the size of response to a single induction shock. There is no change in reflex time.

2. Before spinal transection the individual electric responses show a rapid and marked decrease in size when a series of stimuli are applied with a frequency of four or five a second. After transection this decrease almost disappears; each response is then almost as large as the first of the series.

3. The most striking change in the mechanical response following spinal transection, is a great reduction of the after-discharge, and therefore of the cumulative effect of a rapid series of stimuli.

4. The progressive decrease in the size of the individual responses of a series before transection may be due to the fact that those motor neurones occupied in the after-discharge, being refractory to further excitation, cannot partake in the response to afferent stimulation. It is easier to explain the result this way than by synaptic fatigue, and the explanation harmonizes with the simultaneous reduction of after-discharge and of the decrease in question on spinal transection.

5. The similar decrease in the responses of the afferent paths in the brain stem to a series of stimuli, previously reported, may be similarly explained, and would thus tend to support the view that participation of the higher centers in the after-discharge accounts for the partial abolition of this after-discharge on spinal transection.

6. In like manner the increase in the size of response to a single stimulus following spinal transection may perhaps be explained by the withdrawal of a source of "tonic" activity in the motor neurones.

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CONDITIONS FAVORING MATURATION OF EGGS OF ASTERIAS FORBESII

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Received for publication February 10, 1923

Investigators of marine forms are well acquainted by experience or hearsay with the difficulties involved in experimentation upon the eggs of Asterias forbesii. It has been long known that starfish eggs offer great experimental possibilities due to their unusual sensitivity and to the fact that in eggs removed from the female the entire process of maturation may be observed. However, good lots of eggs maturing in large numbers are often rare, especially during the months of July and August when those who would study them are free to do so. Accordingly any method of treatment which will insure a working percentage of mature eggs should be of value. The purpose of this paper is to present concisely the evidence for the success of certain treatments upon the eggs of Asterias forbesii in an attempt to arrive at a knowledge of the conditions which favor their maturation.

The experiments here described were carried on during July and August, 1922, at the Marine Biological Laboratory at Woods Hole, Massachusetts. This problem was suggested to me by Dr. David H. Tennent of Bryn Mawr College and the work was performed under his direction. I wish to acknowledge his kind coöperation and that of Dr. F. R. Lillie who, as director of the laboratory, placed its equipment at my disposal.

GENERAL METHODS: *1. Setting up an experiment.* In performing the experiments which supply the data for this paper, female starfish were opened and all ten gonads removed, by the use of forceps, to a fingerbowl containing barely enough water to cover them. Other fingerbowls containing 130 cc. of seawater were arranged beforehand; if acid, base, or other reagent was to be used, it was added to that amount of water. The eggs were shaken from the gonads and all refuse removed. The result was a very thick egg suspension. By use of a graduated pipette 2 cc. of eggs were then transferred to each of the fingerbowls previously prepared with different media.

Crowded cultures were made by withdrawing most of the water from the eggs left in the bowl after the 2 cc. lots had been measured out. In such cultures the eggs form a thick layer. It is obvious that crowding was greatest when there was a very large stock of eggs at the beginning. When only small lots of eggs could be obtained, crowding was not so great and therefore less effective in inhibiting maturation.

Throughout the experiments the eggs were distributed in the different cultures as soon as possible after opening the starfish, very little water being used prior to their distribution, in order that the whole process of maturation might take place under the artificial conditions provided.

2. Counting. To determine the effect of various treatments, the eggs were allowed to stand until ample time had been given for maturation; this varied slightly with different lots of eggs and often considerably with the conditions to which they were subjected.

When examination of the eggs was made, a sample was taken from a culture, and the total number of eggs in six microscopic fields, using the low power objective (32 mm.), was counted. At the same time in each field the number of eggs with polar bodies also was obtained. From these two sums the percentage of mature eggs in the culture was determined. In two or three cases counts of four instead of six fields were made, but these were included only when there were several six-count experiments showing the same results from the same conditions. The number of eggs counted necessarily varied in different cultures, for the egg suspensions from which the eggs were measured could not have uniform consistency, since the lots of eggs came from separate females differing in the numbers of eggs which they contained. The average number of eggs observed in each microscopic field was about eighty.

3. Observations. The results of all experiments in the same series (i.e., those conducted with the same reagents or under the same conditions) have been combined, and each series is represented by a line on the graph. In this way the percentages of maturation resulting in different media may be easily compared at once. Space does not permit the publication of the tabular material which is used as a basis for the graph. The number of eggs counted and the percentages determined for each different culture are recorded for each series of experiments. It is hoped that by means of these necessary data and the graph the numerical results of the experiments may be clear, and that a basis may thus be supplied for the conclusions and discussion which are given later.

EXPERIMENTS. The experiments will be taken up as series I, II, III, etc., and the results of each series may be traced readily upon the graph.

Series I. *Effects of crowding, scattering and slight alkalinity.* Six experiments were conducted comparing the effects of these conditions upon the maturation of the eggs. About 2000 eggs were counted in each group designated below.

C—crowded culture—yielding *38.57 per cent* mature eggs.

N—scattered culture, i.e., 2 cc. of eggs in 130 cc. seawater—yielding *79.16 per cent* mature eggs.

NaOH—alkaline culture, i.e., 2 cc. of eggs in 130 cc. seawater plus 1 cc. N/10 NaOH—yielding *91.12 per cent* mature eggs.

The good results obtained when eggs were placed in a large amount of seawater and the still better results following the use of NaOH led to the continued use of crowded, scattered and alkaline cultures in all other experiments for the purposes of comparison. Since no change was made in the preparation of such cultures, they will be designated in all recorded experimental data by the letters C, N and NaOH.

Series II. *Effect of acids.* In comparing the effects of acid and alkaline cultures, three concentrations of NaOH were used, of which the one included in these data (1 cc. N/10 NaOH in 130 cc. seawater) seemed to be the optimum. One of the other two concentrations was weaker than this, while the remaining one was a trifle stronger. Three concentrations of acid were also used, and the middle concentration was chosen here, chiefly because it represented an acidity approximately equal to the alkalinity of its contrasting culture. Results did not vary to any marked degree in the three alkaline cultures, but maturation was inhibited to an increasingly serious extent as the acid concentration was increased.

A. Butyric acid—C_3H_7COOH. Five experiments furnish the percentages for this section of series II. Between 1600 and 2200 eggs were counted for each group of cultures described below.

C—crowded—yielding *13.31 per cent* mature eggs.

N—normal (scattered)—yielding *52.13 per cent* mature eggs.

NaOH—alkaline—yielding *66.15 per cent* mature eggs.

Butyr—2 cc. of eggs in 130 cc. seawater plus 1 cc. N/10 C_3H_7COOH—yielding *28.56 per cent* mature eggs.

B. Acetic acid—CH_3COOH. Five experiments supply the data for the line shown on the graph as series II B. From 2000 to 2300 eggs were counted from each of the four media. Four of the five experiments yielded the same general results shown in the work with butyric acid.

The first experiment of the five, however, showed no lowering of maturation percentages in what were labelled as acid cultures. It is likely that through a mistake the acid was omitted from the acid cultures of that particular experiment; but this is uncertain, and accordingly the data in question are included below and are represented in the same percentages as they are plotted on the graph.

C—crowded—yielding *28.82 per cent* mature eggs.

N—normal—yielding *63.82 per cent* mature eggs.

NaOH—alkaline—yielding *68.82 per cent* mature eggs.

Acet—2 cc. of eggs in 130 cc. seawater plus 1 cc. N/10 CH_3COOH.—yielding *31.83 per cent* mature eggs.

C. Carbon dioxide—CO_2. Six experiments were conducted using the gas from a tank of liquid carbon dioxide. There was no method of gauging the amount used except to time treatments at a constant pressure. The gas was bubbled into the seawater for a definite number of seconds.

The percentage of eggs maturing under crowded conditions was 46 per cent for these six experiments. This was unusually high due to the fact that only small lots of eggs could be obtained, and so crowding could not be so effective. It seemed fair to determine the percentage of mature eggs in *all* crowded cultures prepared during the entire season, and make that the basis for comparison with the carbon dioxide treatments. Nearly 9500 eggs were counted in all the crowded cultures combined, while the counts for the other cultures determined for this series alone approximated 2500 in each case.

C—crowded—yielding *31.64 per cent* mature eggs (based on results of twenty experiments).

N—normal—yielding *69.76 per cent* mature eggs.

NaOH—alkaline—yielding *75.83 per cent* mature eggs.

CO_2—2 cc. of eggs treated with carbon dioxide for 2 seconds—yielding *28.14 per cent* mature eggs.

Treatment with CO_2 for more than 4 seconds was found to inhibit maturation completely in all the eggs. As will be pointed out more fully later, the highly toxic effect of comparatively slight amounts of CO_2 seems to be of specific significance.

Series III. *Effects of oxidizing agents, oxygenation and prolonged agitation.* Four experiments were conducted using potassium permanganate and comparing its effect with those of oxygenation, and protracted agitation. *No crowded cultures were kept.* Oxygenation was accomplished by bubbling oxygen for 15 minutes from a tank of oxygen

into the seawater containing the eggs. Agitation was produced by shaking the eggs continuously for 15 minutes in a flask containing the usual amount of seawater. About 1500 eggs were counted to obtain each percentage shown on the graph for this series.

N—normal—yielding *61.18 per cent* mature eggs.

NaOH—alkaline—yielding *66.56 per cent* mature eggs.

Oxid—2 cc. of eggs in 130 cc. seawater plus $\frac{1}{10}$ cc. N/100 $KMnO_4$—yielding *57.96 per cent* mature eggs.

O—2 cc. of eggs in 130 cc. seawater oxygenated 15 minutes—yielding *78.14 per cent* mature eggs.

Agit—2 cc. of eggs in 130 cc. seawater shaken severely for 15 minutes—yielding *78.88 per cent* mature eggs.

A similar experiment was carried out with sodium hyposulphite with the same result. Oxidizing agents in the medium are less effective than normal seawater in increasing the percentages of maturing eggs. Even when such cultures were agitated in order that a more uniform diffusion of the liberated oxygen might take place, the percentages were not raised by the presence of the oxidizing agent.

Series IV—*Effect of potassium cyanide*—KCN (*not shown on graph*). A few experiments were performed using very dilute solutions of KCN as media for the eggs during their maturation period. The ordinary uncovered fingerbowls were used, so that oxygen was by no means shut off completely. The process of maturation began to the extent that the wall of the germinal vesicle disappeared, but in none of these cultures were eggs found which had given off one or both polar bodies. Observations of these cultures were taken as late as eight hours after the beginning of the experiment.

Normal seawater and alkaline were then compared as recovery media for eggs which had been in the KCN water the length of time usually required for maturation. A small percentage of the eggs completed their maturation.

Recovery in normal seawater—21 per cent mature eggs.

Recovery in NaOH culture—23 per cent mature eggs.

The percentage resulting in the NaOH culture was only slightly above that of normal seawater.

Conclusions: 1. Crowded cultures yield only low percentages of eggs.

2. Acidic conditions prevent maturation to a considerable degree.

3. Oxidizing agents do not raise the percentage of maturation.

4. Substances like potassium cyanide which drive out the oxygen from the medium, inhibit the developmental processes of the egg, so that only a small percentage ever recover when more favorable conditions are substituted.

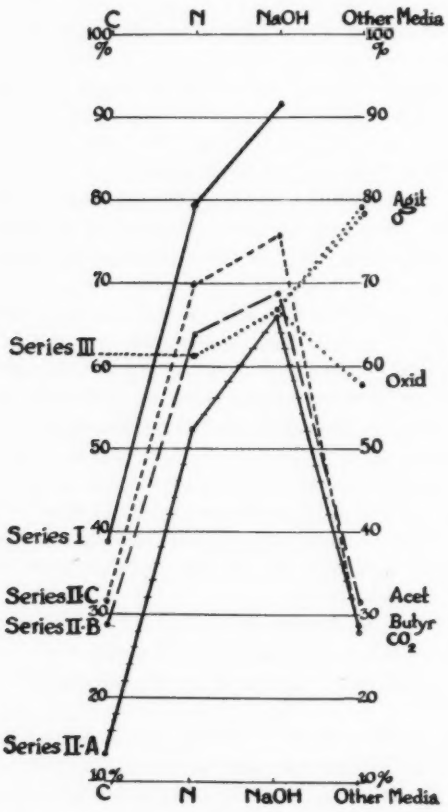

Fig. 1. Graph showing percentages of mature eggs resulting from different treatments.

C, crowded conditions; N, normal scattered conditions; NaOH, alkaline treatment; Butyr, treatment with butyric acid; Acet, treatment with acetic acid; CO_2, treatment with carbon dioxide; Oxid, treatment with potassium permanganate; O, oxygenation of culture; Agit, prolonged agitation of culture.

5. A slight increase in the alkalinity of the normal seawater is a great benefit to the egg while it is in its maturing period.

6. Oxygenation which brings about the rapid movement of the egg as the gas is bubbled into the medium, and prolonged agitation in normal seawater, are about equal in their effect upon the egg. They provide the most favorable conditions.

DISCUSSION OF CONCLUSIONS. In 1912 R. S. Lillie (2) found that refractory eggs obtained late in the season could be rendered more sensitive to experimentation and would yield a higher percentage of maturation if means were used to increase the permeability of the egg membrane either by altering the general state of the colloids in the membrane or by altering only slightly the state of the lipoids alone. He says that "it remains to be determined whether alkali as well as ether (the reagent which he used as a lipoid solvent) can overcome the resistance of such starfish eggs." Lillie suggests the similarity between such eggs and the senescence of cells in general, thinking it possible that there has been a "progressive accumulation or adsorption in the membrane of materials which for some reason are not readily eliminated by the organism."

Several years ago Jacques Loeb (3) performed experiments similar to those recorded in this paper. In 1913 he reported as his conclusions that the "maturation of the eggs of starfish is accelerated by two of the substances present in seawater—the hydroxylions and the oxygen." He found that in an acidified or neutralized medium maturation as a rule did not take place, while the rapidity with which maturation occurred increased within certain limits with the concentration of the hydroxylions. The necessity of a good oxygen supply was proved, according to Loeb, by the fact that eggs piled in a heap in a crowded culture matured with difficulty. He also conducted experiments with potassium cyanide showing that eggs failed to mature in a medium where oxygen had been driven out. The results of our experiments confirm Loeb's in many ways. Our observations however were made from the point of view of the number of eggs which finally matured rather than the lengths of time required under different conditions. With the exception of the treatments following KCN water, and the higher acid concentrations, maturation was seldom noticeably delayed by the different media. The percentages were seriously lowered indicating that maturation was either partially or completely inhibited. In most cases of partial inhibition, the eggs began the maturing process but never finished, no matter how long the culture was allowed to stand.

While Loeb and R. S. Lillie have found the use of various chemicals helpful in securing a high percentage of mature eggs of Asterias, A. P. Mathews and E. B. Wilson (5) found mechanical agitation to be of great value. Mathews (4) regarded the process as one of oxidation, believing that the agitation of the egg helped to rupture the wall of the germinal vesicle where it came nearest the surface of the egg, thus setting free into the cytoplasm an oxidase which facilitated the action of atmospheric oxygen on the egg.

The experiments recorded in this paper have led to our belief that *the whole problem of the maturing egg is one of respiration—oxygen must have access to the egg, and carbon dioxide given off at the surface of the egg must be eliminated from the egg's immediate surroundings.* These factors in respiration are offered as the basis for the explanations of the six points given as conclusions:

1. A crowded culture provides in any case a lack of uniformity in the environment of the egg. The eggs lie in a thick layer at the bottom of the fingerbowl. The eggs at the bottom of this layer are saturated with their own carbon dioxide and are far removed from any supply of oxygen. Those at the top of the layer come into more complete contact with the seawater, passing off their carbon dioxide where diffusion, dilution, and to a slight extent neutralization take place very slowly. The degree of saturation of carbon dioxide is bound to be lower there than at the bottom of the layer, and consequently there is opportunity for the egg to take up oxygen for its metabolic activity. The eggs on the top layer next to the seawater are undoubtedly the ones which furnish the small percentage of mature eggs found in crowded cultures.

2. Acidic treatments lower the percentage of maturation because they cannot provide for the elimination of carbon dioxide which is being given off constantly. Plenty of oxygen may be present but it cannot get at the egg in an acid culture because the carbon dioxide immediately surrounding the egg admits of no real exchange of gases. It is difficult to say whether acids have any harmful property in themselves; perhaps their ill effect is due solely to their inability to counteract the saturated condition resulting from the constantly increasing amount of carbon dioxide. In any case many eggs are apparently poisoned by the waste product of their metabolism which is not neutralized or conducted away by acids when they are introduced into such a medium.

3. Oxidizing agents do not favor maturation. This may be due to the superficial nature of oxidation as carried on by the reagent. It is likely that diffusion is hindered mechanically by the reduction product which coats the egg, preventing rather than aiding respiration.

4. The explanation of the results of treatments with potassium cyanide is an obvious one. Oxygen was cut off and for such a long time that when conditions were made more favorable, only a few eggs went on and matured normally.

L. H. Hyman (1) in 1916 reported that in dilute solutions the primary effect of potassium cyanide is the stimulation of the oxygen consumption followed almost immediately by a depression in the oxygen consumption which is the more marked the higher the concentration of potassium cyanide used. Here then is a possible explanation for the dissolution of the germinal vesicle under conditions so adverse that polar bodies were never thrown off.

5. Slight alkalinity probably aids the process of maturation in two ways:

a. It probably helps in getting carbon dioxide to the outside surface of the egg by increasing the permeability of the egg membrane.

b. After the carbon dioxide gets into the medium the resulting acid condition is neutralized by the presence of the alkali, so that more and more carbon dioxide can be eliminated without saturation of the medium which finally stops both the intake of oxygen and the elimination of waste.

6. Oxygenation and protracted agitation have been shown to be practically the same in the condition which they provide for the egg. They are essentially the same process as far as their usefulness to the egg is concerned. Excess oxygen is introduced in the one case and not in the other. Apparently all the oxygen which the egg can use is present in the medium of normal seawater, if the eggs are not crowded. Diffusion is favored in both the oxygenation and the protracted agitation, not by rendering the membrane more permeable, but by bringing the egg into new places in the medium where carbon dioxide can be given off into an unsaturated environment. The process of agitation of eggs in a large amount of seawater is reproducing in the laboratory the very conditions which the egg would normally meet in its natural environment. The good results are to be expected. On the other hand, in accordance with the explanation given here or some other, a slightly alkaline medium produces an effect nearly as good as that found in the neutral surroundings of the egg.

SUMMARY

1. The problem of maturation in the eggs of Asterias forbesii is essentially a problem of respiration. Ample opportunity must be given

for the elimination of carbon dioxide and for the intake of oxygen. Unless the environment provides for *both* these factors in respiration, the eggs do not mature in large numbers.

2. Experiments are recorded which show that respiration and therefore maturation are *not* favored:

a. When eggs are crowded together in great numbers with a small amount of seawater;

b. When the medium of the seawater has been rendered acidic;

c. When oxygen is driven out of the medium by the use of potassium cyanide.

3. Experiments are recorded showing that oxidizing agents such as potassium permanganate do not affect the percentage of maturation to any marked degree, when they are introduced into the seawater in which the eggs are placed.

4. Experiments are recorded showing that the maturation of large numbers of eggs is brought about:

a. When the eggs are placed in large quantities of normal seawater (fifty or sixty times as much seawater as the measured quantity of eggs);

b. When a small amount of sodium hydroxide is added to the seawater before the eggs are transferred to that medium (about 2 cc. N/10 NaOH should be added to every 250 cc. of seawater used—not more than 4 cc. of eggs should be placed in this amount of seawater);

c. When eggs are severely agitated in large amounts of seawater for several minutes.

5. Since the method of severe agitation may not be practical in all laboratories, the use of sodium hydroxide in the proportion given above is suggested as a means of securing at any time during the season a good percentage of mature eggs.

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THE REGULATION OF RENAL ACTIVITY

VIII. The Relation between the Rate of Urea Excretion and the Size of the Kidneys[1]

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Received for publication February 14, 1923

Some observations on the relation between the size of the kidney and the rate of urea excretion were published in 1918 in an earlier paper of this series (1). Since the average rate of urea excretion in man was found to be about 30 times greater than the average rate observed at the same blood urea concentration in rabbits, and since there is approximately 30 times more renal tissue in a man than in a rabbit, it was suggested that the rate might vary directly with the size of the kidney. But under the experimental conditions we then observed it was not possible to demonstrate the effect on the rate of urea excretion of such differences in the amount of renal tissue as occur in adult members of the same species because any constant influence which these minor structural differences might have exerted was obscured by considerable variations in the rate of urea excretion due to factors of an unknown nature. We later found certain special conditions under which the effect of these disturbing factors was eliminated and the rate in any one subject was then found to vary only with the blood urea concentration so that the ratio: $\frac{\text{rate of urea excretion}}{\text{blood urea concentration}}$ was constant when the factor of kidney size was constant, as when the ratio measurements were made on the same individual, but varied when the factor of kidney size varied, as when the measurements were made on different individuals (2). Since it appeared that under these conditions the differences in the value of the ratio were related, roughly at least, to differences in body weight and so presumably to differences

[1] This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

in the weight of the kidneys, it seemed probable that the rate of urea excretion, though at times considerably modified by a great variety of factors, was controlled by only two factors of constant and primary importance, the concentration of urea in the blood and the amount and quality of the urea-secreting tissue of the kidney.

The most direct evidence of a relation between the size of the kidney and the rate of urea excretion would be obtained by comparing the value of the ratio: $\frac{\text{rate of urea excretion}}{\text{blood urea concentration}}$ with the weight of the kidneys of normal subjects. This is obviously impossible with human subjects and unfortunately the conditions under which the ratio becomes a constant for each individual can be attained only in subjects who can coöperate by drinking urea solutions and water, who can void urine spontaneously at suitable intervals and who are not disturbed by the removal of blood. In rabbits it is necessary to use a stomach tube and to catheterize and wash the bladder, and possibly on account of these procedures measurements of the ratio on the same individual show considerable variations. But if there is a direct relation between the magnitude of the ratio and the size of the kidney it should be demonstrable in rabbits of widely varying size, although only an approximate agreement can be expected.

Twenty-five male rabbits varying in weight from 453 to 3690 grams were used. After abstinence from food for 18 hours, 1 gram of urea per kilo body weight was given in 4 per cent solution by stomach tube, and an hour later the tube was again introduced and 15 cc. of water per kilo body weight were administered. At least 1½ hours after giving the urea the bladder was catheterized and washed. At approximately hourly intervals thereafter three collections of urine were made by catheterization and after each collection the bladder was washed out. Samples of blood were obtained from the marginal ear vein at the middle of each of these three periods of urine collection. The urea in the urine and the blood was determined by the urease method and the average ratio: $\frac{\text{urea in 1 hour's urine}}{\text{urea in 100 cc. of blood}}$ was calculated. The following is a typical protocol

Rabbit 16. Weight, 2440 grams

8:00 a.m. Given 61 cc. of 4 per cent urea solution in water by stomach tube.
9:07 a.m. Given 37 cc. of water by stomach tube.

The times given in the following table are those at which the washing of the bladder was completed and the midpoint in the few minutes required for the collection of blood.

TIME	URINE UREA	TIME	BLOOD UREA	RATIO $\frac{\text{URINE UREA}}{\text{BLOOD UREA}}$
	mgm. per hour		*mgm. per 100 cc.*	
9:49–10:48	324	10:17	116	2.79
10:48–11:48	295	10:18	113	2.61
11:48–12:54	268	12:18	104	2.58
Average				2.66

Four such experiments were carried through on different days on rabbits 13 and 21, three on 12, 15, 16, 18 and 22, and two on the remainder. At the conclusion of the last experiment the animals were killed, and the kidneys at once removed and weighed. They were later examined microscopically by Dr. Jean Oliver who reported that they were all normal or almost normal except for the kidneys of rabbits 24 and 25 which showed extensive spontaneous nephritis. The weights of the normal kidneys are compared with the average ratios in table 1, and all the observations are charted in figure 1.

These results indicate that in rabbits there is an approximately direct relation between the magnitude of the ratio and the weight of the kidney. It is very probable that a better agreement would have been found if the experiments could have been carried out on human subjects in whom a much higher degree of constancy in the value of the ratio for each individual can be attained. But in these observations on rabbits the only ratios which deviate markedly from the average trend are those on numbers 24 and 25, shown as circles in figure 1. In these animals the ratio is less than might have been expected from the weight of the kidneys, but it was in their kidneys only that a considerable part of the urea-secreting tissue had been destroyed and replaced by fibrous tissue. The fourth column in table 1 gives the ratio divided by the weight of the kidneys in grams, or the ratio for each gram of renal tissue. The average is 0.176 per gram. If the mechanism of urea excretion in the rabbit is comparable with that in man, the ratio in man should be about 300 times greater than 0.176, since man has about 300 grams of renal tissue. One would therefore expect a ratio of about 52.8. The average ratio found in 31 normal adult men was 50.4 (3), which is an agreement sufficiently close to

warrant the deduction that weight for weight the kidney of the rabbit is as active in the excretion of urea as the kidney of man.

In adult normal human subjects the value of the ratio varies somewhat in each individual. The coefficient of variation was 13.1 per cent with an average ratio of 50.4. A considerable part of this variability

TABLE 1

Relation between the weight of the kidneys and the ratio: $\frac{\text{urea in 1 hour's urine}}{\text{urea in 100 cc. of blood}}$

RABBIT NUMBER	WEIGHT OF KIDNEYS	RATIO: $\frac{\text{URINE UREA}}{\text{BLOOD UREA}}$	RATIO: $\frac{\text{URINE UREA}}{\text{BLOOD UREA}} \div$ WEIGHT OF KIDNEYS
	grams		
1	5.76	0.46	0.08
2	6.23	0.95	0.15
3	7.01	0.62	0.09
4	7.35	1.12	0.15
5	8.83	1.56	0.18
6	9.23	1.93	0.21
7	10.19	1.81	0.18
8	11.28	2.07	0.18
9	11.79	2.28	0.19
10	11.81	2.62	0.22
11	12.02	2.01	0.18
12	12.10	2.52	0.21
13	13.40	2.67	0.20
14	13.63	2.42	0.18
15	13.84	2.04	0.15
16	14.00	2.51	0.18
17	14.43	2.28	0.16
18	14.69	3.53	0.24
19	17.30	3.45	0.20
20	19.38	4.12	0.21
21	21.20	3.19	0.15
22	23.72	4.03	0.17
23	24.59	4.79	0.20
Average per gram renal tissue.....			0.176

is undoubtedly due to variation in the amount of renal tissue in different normal individuals, and if some index of this physiological variation could be obtained a correction might be introduced and a better standard of normality for ratio values would be obtained. This is a matter of some practical importance because ratio measurements on patients whose kidneys have been partially destroyed by disease

may be used as a method for estimating the amount of urea-secreting tissue they still possess. The validity of a correction based on body weight is open to question, for presumably the amount of renal tissue bears some relation to the amount of work it does and the body weight might be increased by adipose tissue without any appreciable increase in the work of the kidney. Further, Stewart (4) has found that in dogs the weight of the kidneys is more closely related to body surface

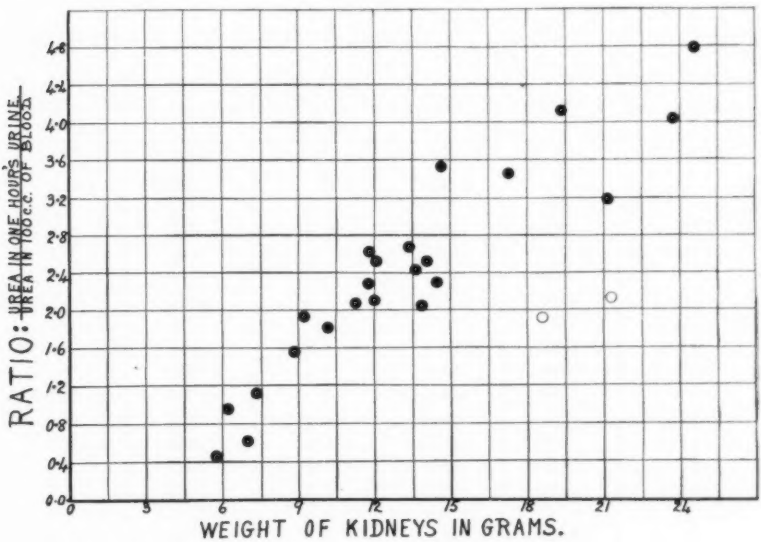

Fig. 1. The solid dots indicate the relation between the ratio and the weight of the kidneys in the animals whose renal tissue was normal or almost normal. The circles show the relationship in rabbits 24 and 25 whose renal secreting tissue had been in part destroyed by spontaneous nephritis.

than to body weight but as he was able to make only a small number of observations, we thought it would be worth while to investigate the relationship in rabbits. As soon as the animal was killed and the kidneys weighed, the skin was removed, cut up into sections so that each could be flattened out on a sheet of paper, and outlined in pencil. A planimeter was run round these outlines and the total skin area in square centimeters obtained. The body weights are the averages of several weighings made while the ratio measurements were being

made. In table 2 the kidney weights, body surfaces and body weights are given.

The body surface shows a more constant relation to the kidney weight than the body weight and this result in combination with Stewart's findings has led us to correct ratios obtained in patients by multiplying the observed ratio by the normal body surface divided by the body surface of the patient as estimated from his height and weight.

TABLE 2

The relation between the weight of the kidneys and the body surface and the body weight

RABBIT NUMBER	WEIGHT OF KIDNEYS	BODY SURFACE	BODY SURFACE ÷ WEIGHT OF KIDNEYS	BODY WEIGHT	BODY WEIGHT ÷ WEIGHT OF KIDNEYS
	centigrams	*sq. centimeters*		*grams*	
1	576	499	0.9	453	0.9
3	701	838	1.2	830	1.2
4	735	811	1.1	880	1.1
6	923	1423	1.5	1593	1.7
7	1019	1321	1.3	1283	1.3
9	1179	1520	1.3	2279	1.9
10	1181	1457	1.2	1761	1.5
11	1202	1271	1.1	1938	1.6
12	1210	1408	1.2	1830	1.5
13	1340	1685	1.3	2503	1.9
14	1363	1443	1.1	2400	1.8
16	1400	1578	1.1	2610	1.9
17	1443	1548	1.1	2405	1.7
18	1469	1680	1.1	2534	1.7
19	1730	1668	1.0	2767	1.6
21	2120	2132	1.0	3380	1.6
22	2372	2428	1.0	3690	1.6

It has already been shown (5) that our previous tentative conclusion that the rate of urea excretion is limited by the size of the kidney when the blood urea concentration reaches very high levels (1) was mistaken. Under suitable conditions the rate of urea excretion continues to increase with the every increase in blood urea concentration until general toxic symptoms develop, so that the ratio between the rate of urea excretion and the blood urea concentration remains approximately constant at all practicable levels of blood urea concentration. But the order of magnitude of this ratio is regulated directly by the size of the kidney.

CONCLUSION

The rate of urea excretion, though influenced within certain limits by a great variety of factors, is primarily controlled only by the concentration of urea in the blood and the amount of urea-secreting tissue in the kidney, so that under certain conditions when the effect of variation in blood urea concentration is eliminated the rate of urea excretion varies in direct proportion to the weight of renal tissue.

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A STUDY OF REVERSAL EFFECTS OBSERVED IN EXPERIMENTS WITH SODIUM CITRATE OR ALLIED SALTS AND AUTONOMIC DRUGS

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Received for publication February 15, 1923

It was shown in previous communications (1), (2) from this laboratory that sodium citrate injected into different animals caused a fall of blood pressure, stimulated respiration and increased the motor functions of the intestine. Evidence was also adduced indicating that the contractions of the intestine were due to the effect of citrate on the termination of the vagal fibers. In the course of these observations on the point of action we noticed that citrate given after atropine sometimes caused relaxation of the intestine and diminished or abolished the rhythmic contractions. A further study of this phenomenon was therefore undertaken and numerous experiments were performed with citrate on animals that had been given atropine and pilocarpine. These observations were extended to the action of other calcium-precipitating salts such as tartrate, oxalate and fluoride when administered in conjunction with the alkaloids. Tests were also made on the effects of adrenalin and citrate. The results of the present investigations were largely obtained by experiments on cats but occasionally dogs also were used. All the experiments were performed on completely anesthetized animals using urethane dissolved in water and given by mouth through a stomach tube, or a 5 per cent solution in normal saline was injected slowly into the femoral vein after preliminary ether anesthesia. This combination had the advantage of greatly diminishing the amount of urethane required to keep the animals in a proper state of narcosis and thus avoiding excessive depression of respiration and of the circulation which it produced, especially when given in large quantities. The methods of recording respiration, blood pressure and intestinal movements were the same as those employed in the previous communications referred to.

Experiments with sodium citrate. That the response of the intestine to citrate may be completely changed when given after atropine has been indicated above. A number of trials were made later with the two drugs confirmed our previous observations. We found that the intravenous injection of sodium citrate, especially when large doses were

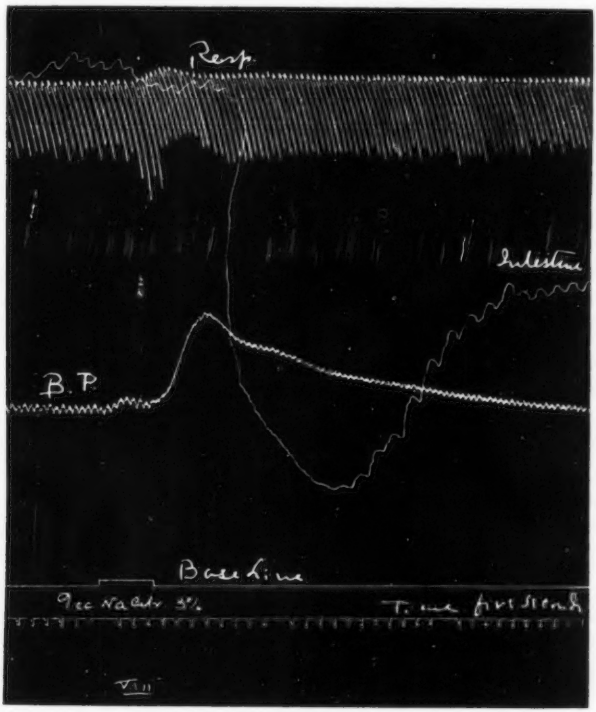

Fig. 1. Dog, weight 4.5 kilos. Urethane anesthesia. Reversal effects on blood pressure and intestinal contractions produced by citrate after atropine and pilocarpine.

given, produced marked depression of the motor functions of the intestine if atropine had been administered previously. Similar effects on the intestine followed the injection of sodium citrate after atropine and pilocarpine and even after pilocarpine alone. The reaction of the circulation to citrate under these conditions was likewise different from

that observed when the salt was administered to animals that had not received the alkaloids. Typical experiments illustrative of the results obtained are presented, the protocols being considerably abbreviated by omitting unessential details.

A cat weighing 3 kilos received intravenously four injections of 60 mgm. per kilo each of sodium citrate. This produced the usual changes, namely, a fall of blood pressure, increased respiratory activity and stimulation of the intestinal contractions. A dose of 0.02 mgm. atropine (as sulphate) given after the fourth dose of citrate had no effect on the next injection of citrate, but when the same amount of atropine was given again, subsequent injections of citrate failed to stimulate intestinal motility. There was, on the contrary, relaxation of the intestine but the effects on the blood pressure and respiration were the same as those previously observed. After an interval of a few minutes 3 mgm. of pilocarpine were injected which resulted in strong regular movements of the intestine, though not as vigorous as those generally observed when pilocarpine alone is given intravenously. Upon the injection of sodium citrate the intestine was promptly relaxed, tonus was considerably decreased while the rhythmic contractions became weak and irregular. This condition lasted about 3 minutes before any sign of improvement was observed. The results of this experiment show that sodium citrate may cause excitation as well as inhibition of the intestine. A similar experiment was then performed on a dog, likewise under urethane anesthesia. Sodium citrate in amounts of 30 and 60 mgm. per kilo stimulated the intestinal contractions and respiration, but no appreciable changes could be noticed in the circulation. When the same amounts of citrate were injected after the administration of pilocarpine, respiration and the intestine were stimulated and blood pressure showed some elevation. Later sodium citrate was injected after atropine. Intestinal tonus was decreased also in this case, respiration became deeper and slower and blood pressure fell. That sodium citrate may cause relaxation of the intestine was observed again in another experiment. The first dose of 0.2 mgm. atropine injected into a dog weighing 5.6 kilos failed to exert any influence on the subsequent injections of sodium citrate but the second dose of 0.2 mgm. of the alklaloid proved more effective. The injection of 60 mgm. sodium citrate now produced relaxation of the intestine as in previous experiments, but neither blood pressure nor respiration was appreciably affected. Since the intestine no longer contracts but may on the contrary become relaxed by sodium citrate when injected after pilo-

carpine, we directed our attention once more to the combined effect of atropine and pilocarpine on its action. A brief protocol of one of these experiments follows:

A dog weighing 4.5 kilos received 30 mgm. sodium citrate per kilo. The usual response occurred promptly following this injection. Several doses of sodium citrate were given later after the administration of atropine, and rather large doses of pilocarpine. As before, the intestine was depressed, the relaxation increasing with the size of the dose, but circulatory changes were also reversed. Blood pressure was considerably elevated, the rise bearing a striking resemblance to that observed after the injection of adrenalin. In some experiments when sodium citrate was injected after atropine and fairly large doses of pilocarpine the rise in blood pressure varied between 200 and 250 per cent. In another series of experiments we tested the effect of citrate after pilocarpine alone. The results obtained have shown a similar tendency to that already described when citrate was administered after the injection of both alkaloids. Some of the experiments may be briefly described. A dog weighing 3.2 kilos received different amounts of sodium citrate intravenously. The contractions of the intestine as well as respiration were stimulated, but practically no effect was observed on blood pressure. Small doses of pilocarpine were then injected intravenously at various intervals, and after a total of 1 mgm. of the alkaloid had been given more sodium citrate was injected. The effects on the intestine and on respiration were the same as after previous injections of the salt in this experiment but there was a rise of blood pressure of 25 mm. (about 22 per cent) followed by a gradual fall to the normal level. Sodium citrate after pilocarpine alone also produced reversal effects in cats. Three cubic centimeters of 3 per cent sodium citrate were injected into a cat weighing 2.3 kilos, no previous injections of pilocarpine or atropine having been made. Immediately after the introduction of the salt there was greatly increased tonus and stimulation of the rhythmic contractions of the intestine. Blood pressure fell about 15 mm. (180 to 165), respiration became more frequent and the amplitude was increased. The injection of sodium citrate was repeated after 1 mgm. of pilocarpine per kilo. A persistent elevation of blood pressure of 33 mm. (about 25 per cent) was now observed which was preceded by a transitory fall. There was also relaxation of the intestine which was of short duration but was nevertheless very distinct. Several injections of sodium citrate were made again at different intervals after the additional injection of pilocarpine. While the rise of blood pressure

improved with each successive dose the initial fall was much less prominent after later injections. Depression of intestinal activity was not as marked, however, as after previous injections of citrate but its presence could not be mistaken. Another illustration of reversal effects observed after sodium citrate was furnished by the next experiment in which the conditions were practically the same as in the one described. It should be added that both vagi were cut in these two experiments as soon as anesthesia was complete and before the injections were begun. The injection of 4 cc. 3 per cent sodium citrate into a cat weighing 4 kilos produced a slight elevation of blood pressure.[1] There was hardly any effect on respiration or on the intestinal movements. After a lapse of 1 minute, the injection of the same dose was repeated. Respiration again showed very little change, blood pressure fell 10 mm., but the intestine showed violent activity. When sodium citrate was given after the injection of 2 mgm. pilocarpine, relaxation of the intestine occurred and blood pressure fell promptly but soon rose 20 mm. higher than before the injection. Similar reversal effects were observed when the injections of pilocarpine and citrate were repeated later in this experiment. The effect was very striking when sodium citrate was injected in two cats which received large amounts of pilocarpine. Doses of 2 to 5 mgm. pilocarpine were given until a total of 30 mgm. of the alkaloid was introduced. About 40 mgm. sodium citrate per kilo produced in one experiment a rise of blood pressure of nearly 40 mm. or about 38 per cent. The effect was less pronounced in the other experiment and was preceded by a slight fall of blood pressure which was probably of cardiac origin but it persisted without change for 2 minutes. Respiration was slightly stimulated and intestinal tonus was depressed for a brief period. It is evident from the results described that atropine and pilocarpine reversed the effect of sodium citrate, the modifying action however being different for each alkaloid. Atropine either abolished the stimulating action of sodium citrate on the intestine or converted excitation into inhibition while blood pressure and respiration showed the usual effect of citrate. Pilocarpine has in most instances brought about a complete reversal of the effect of sodium citrate on blood pressure as well as on intestinal motility, though in some instances only one or the other was reversed. But in no case did we obtain evidence of reversal effects on respiration. Tests were also made in which the order of injection was inverted as it was within the realm of probability that it was the pilocarpine which caused the

[1] Adrenalin was injected 6 minutes before the citrate.

reversed action since it was undoubtedly present in the body at the time sodium citrate was injected. This suggestion was made highly probable in view of the modifying action of sodium citrate on the effect of mercury, which will be discussed later. When pilocarpine was injected after citrate, reversal effects did indeed manifest themselves, as a rise in blood pressure occurred and in several instances there was also depression of the intestine. It may be remarked, however, that these reversal effects occurred much less frequently than when citrate was injected after pilocarpine. In this connection we wish to state that these experiments were controlled by repeated injections of pilocarpine alone and the effect of these on the circulation and the intestine was observed. We found that even large amounts injected in doses of 2 to 5 mgm., in cats weighing between 2 and 3 kilos, caused more rapid heart action and only very moderately increased the blood pressure, whereas the elevation of blood pressure when pilocarpine was injected after citrate was 27 to 33 per cent.

Experiments with sodium tartrate. It has been maintained by a number of observers, especially by Sabbatani (3) and more recently by Brailsford Robertson (4), that citrate, oxalate and tartrate owe their action to disturbance of the calcium mechanism. Their pharmacological effects should therefore be the same or at least closely similar. Experiments were, therefore, carried out with sodium tartrate, oxalate and fluoride to ascertain whether their behavior in the body when injected after pilocarpine was the same or similar to that observed after sodium citrate. Since the effect of these salts on the circulation and intestinal motility has not been determined before, we first made a study of the nature of the response when they were given intravenously. It may be briefly stated that sodium tartrate was found to be much less active than citrate, as much larger doses were required to produce the same changes either in the circulation, respiration or in the contractions of the intestine. Whereas a dose of 20 to 30 mgm. sodium citrate per kilo caused greatly increased movements of the intestine and depression of the circulation, the amount of sodium tartrate necessary to produce the same results on the intestine was about three times as much while still larger doses had to be injected to cause a moderate fall of blood pressure or stimulate respiration. The reaction obtained in one experiment with 30 mgm. sodium citrate was approximately the same as after the injection of 150 mgm. sodium tartrate. This would indicate that the difference was quantitative and observations on the behavior of tartrate after atropine have shown that the point of action

of this salt in the intestine was probably not different from that of citrate. After the injection of 0.16 mgm. atropine into a cat weighing 1.4 kilos, sodium tartrate still produced augmentation of intestinal movements, but less than before the alkaloid was given and after a long latent period. In another experiment, however, a large dose of sodium tartrate injected into a cat weighing 2.3 kilos which had received 0.2 mgm. atropine and 10 mgm. pilocarpine produced only a slight increase of intestinal tonus. Tartrate probably acts, therefore, similarly to citrate attacking the vagal terminations in the intestine. When tartrate was injected after pilocarpine and atropine or after pilocarpine alone moderate depression of intestinal contractions was observed in some cases while no reaction was obtained in others. It was different in the case of the circulation as is shown in the following experiments. In one of these experiments blood pressure rose from 55 to 85 mm. when 10 cc. 3 per cent sodium tartrate were injected 4 minutes after the administration of 4 mgm. pilocarpine into a cat weighing 2.8 kilos. Moreover, the elevation of blood pressure persisted. Relaxation of the intestine was also observed but this was transitory. Sodium tartrate (9 cc.) injected before pilocarpine caused a slight increase of blood pressure and moderate stimulation of the intestine. In another experiment 11.5 cc. 3 per cent sodium tartrate were given to a cat weighing 2.3 kilos. Blood pressure fell slightly but respiration and the intestine were stimulated, the effect lasting in each case about 2 minutes. The same dose of tartrate when given later after the injection of pilocarpine and atropine increased blood pressure from 50 to 110 mm. for a period of more than 10 minutes. No change could be observed however in the intestine or respiration. The injection of tartrate was repeated a number of times but the same effect was always produced. Similar results were obtained in other experiments although the results were not as pronounced as in those described. Particularly interesting were the observations on the changes produced by pilocarpine in animals that had previously received sodium tartrate. In some instances blood pressure was increased more than 100 per cent. We observed in one experiment a rise of blood pressure from 50 to 115 mm. which lasted 13 minutes when 5 mgm. pilocarpine were injected into a cat weighing 2.3 kilos. These observations thus afford another instance of similarity of action of tartrate and citrate. But it might be added that the reversal effects on the circulation were more striking with pilocarpine after tartrate than after citrate.

Experiments with sodium oxalate. The observations were made on cats and dogs. The first injection of 30 mgm. sodium oxalate per kilo produced in one dog a transitory rise of blood pressure and increased intestinal tonus. Repetition of the dose was followed by a preliminary fall succeeded by a considerable elevation of blood pressure which was

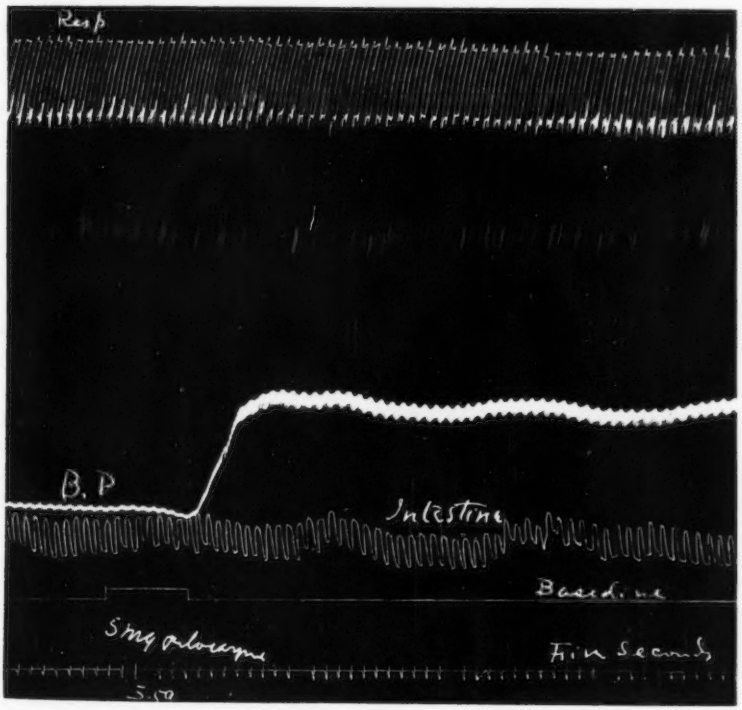

Fig. 2. Cat, weight 2.3 kilos. The rise of blood pressure shown in figure lasted 13 minutes. Considerable amounts of sodium tartrate and some atropine given before pilocarpine.

of short duration while intestinal tonus was depressed and the rhythmic movements were inhibited. The same amounts per kilo of body weight given to another dog caused a transitory fall of blood pressure of about 8 mm. and slightly accelerated respiration for a brief period. These observations were thereafter made entirely on cats as we found that they

were better adapted for our purpose though they were less resistant to oxalate than dogs. Small doses were therefore injected, especially since these had to be repeated a number of times for the study of reversal effects. The response to oxalate likewise varied in these animals causing increased movements of the intestine in some individuals and no effect in others. We also made the very interesting observation that under certain conditions a small dose of oxalate may even cause relaxation of the intestine resembling that which occurs after the injection of adrenalin. Thus in one experiment the intestine had been contracting forcibly for 18 minutes finally going into a condition of spasm. After this cramp-like state continued for some time 28 mgm. sodium oxalate were given intravenously. This produced depression of tonus, the intestine remaining in this condition until pilocarpine was injected 9 minutes later. The circulation was depressed after several small doses while respiration was moderately stimulated. Injections of pilocarpine showed some effect on the subsequent administration of oxalate but the evidence of reversal changes was by no means striking. We noticed in one case that oxalate after pilocarpine failed to depress the circulation. More satisfactory results, however, were obtained in another experiment. In this case 34 mgm. pilocarpine were given in divided doses in one hour to a cat weighing 4 kilos. Five minutes after the last injection of pilocarpine 3 cc. 2.8 per cent sodium oxalate were given intravenously. This produced a persistent rise of blood pressure of 25 mm. or about 16 per cent. The same dose was given 18 minutes later and again elevation of blood pressure of 25 mm. occurred. The immediate effect of the injection however was a fall of blood pressure less than 10 mm. when the entire amount was introduced. Recovery then set in and within 1 minute blood pressure reached the height indicated. No effect was produced by oxalate on the intestine under these conditions. After the administration of several doses of pilocarpine the intestine became tonically and permanently contracted, oxalate producing no change in its condition. Hence it may be concluded that oxalate alone after pilocarpine may cause reversal effects on the circulation and not on the intestine. But it may be further observed that the circulation also soon fails to manifest these changes. When 14 mgm. oxalate per kilo were injected making a total of 56 mgm. per kilo, heart action showed signs of marked depression even after large amounts of pilocarpine.

Experiments with sodium fluoride. The results obtained with this salt were somewhat similar to those described in the experiments with

the other calcium-precipitating compounds. Experiments were performed on four cats to which doses of 10 to 15 mgm. of fluoride per kilo were administered intravenously at frequent intervals. These amounts caused a fall of blood pressure of a few millimeters and slight stimulalation of intestinal tonus. In one of the experiments 10 mgm. sodium fluoride per kilo produced little change in the circulation, intestinal movements or respiration. But when several doses were given the effect was much greater. Blood pressure fell from 140 mm. to 115 mm. immediately after the injection and gained only 5 mm. in height during the next 3 minutes. The effect on the intestine was likewise prolonged but was never as marked as after sodium citrate and respiration was moderately increased in frequency. These amounts of fluoride injected after 3 to 5 mgm. pilocarpine per kilo did not produce marked changes, but in one experiment in which a large amount of pilocarpine was given in divided doses the injection of 9 cc. of 0.7 per cent fluoride caused an elevation of blood pressure from 111 mm. to 136 mm. in 7 minutes. The intestine was not affected, however, by this injection of fluoride. As stated above, one-third the amount of fluoride injected in untreated animals caused a considerable depression of the circulation. The fall of blood pressure measured in millimeters of mercury was about equal to the rise which occurred when three times the amount was given after repeated injections of pilocarpine. This shows that reversal effects may also be obtained when sodium fluoride is given to cats that had received pilocarpine. In none of the experiments however could evidence of reversal effects on the intestine be obtained.

Discussion. Of the numerous possibilities which have suggested themselves for the explanation of the reversal phenomenon described in the preceding pages we will present a few which, we believe, merit consideration. The relaxation of the intestine with coincident diminution of the rhythmic movements following the injection of sodium citrate after atropine may be explained by the observation which we made that stimulation of the peripheral end of the vagus after atropine caused marked relaxation of the small intestine. Starling (5), it may be recalled, describes similar effects on the esophagus and cardiac sphincter when the vagus is stimulated after atropine. We have shown that sodium citrate stimulated the intestinal contractions by affecting the peripheral vagus mechanism. It may be observed, therefore, that when the motor endings have been paralyzed, the terminal organs of the inhibitory fibers still respond to citrate under these conditions. It is much more difficult to account for the effect observed when sodium

citrate and the allied salts are given after pilocarpine. The following observations seemed to offer some suggestion as to the possible mechanism involved in these reversal phenomena. The simultaneous rise of blood pressure and depression of the intestine indicated that increased activity of the adrenals might be a causal factor. According to Dale and Laidlaw (6) pilocarpine may stimulate the secretion of the adrenals. Corroborative evidence of this was also furnished recently by Edmunds (7). This amount, however, may be insufficient to cause any changes in the circulation or intestinal motility in normal animals. It may be recalled that Chiari and Froelich (8) observed that oxalate increases the response of the sympathetic system to autonomic drugs. It is possible, therefore, that citrate which inactivates calcium would produce a similar effect. Hence the combined action of pilocarpine and the calcium precipitating salts would result in producing the effects which we observed. To test this hypothesis the adrenals were extirpated and pilocarpine as well as citrate was injected. Six experiments were performed but the results were negative. Indeed the rise of blood pressure was in some experiments distinctly greater when citrate preceded by pilocarpine was injected after, rather than before, removal of the adrenals. Other explanations were then sought and will now be discussed. If the rise of blood pressure and inhibition of intestinal movements were not caused through the intermediation of adrenal activity, perhaps pilocarpine was still the cause of the reversal effects, for it is conceivable that it might have been transformed into a sympathetic stimulant. Indeed, as pointed out recently by Sollmann (9), evidence is not wanting that parasympathetic drugs may become sympathetic stimulants and vice versa. It has been shown that epinephrin in the presence of calcium deficiency may stimulate the parasympathetic endings and that in certain localities pilocarpine does stimulate the terminations of the sympathetic fibers. The reversal phenomena described in the preceding pages may be due, therefore, to transposed action of pilocarpine brought about by the influence of the various salts employed. But according to this explanation pilocarpine injected after citrate or the other salts should likewise cause reversal phenomena. We have shown that blood pressure is indeed considerably elevated by pilocarpine when injected into animals that have received citrate or tartrate but that the effect did not occur very often. Besides we very seldom observed relaxation of the intestine when pilocarpine was injected after citrate or tartrate. Hence transposition of action does not satisfactorily explain the reversal changes observed,

and an entirely different process was therefore probably at work in causing the manifestation of the phenomena described. In this connection the following observations are suggestive. As reported in a recent publication from this laboratory (10), it was found that small doses of mercury, when given intravenously a few minutes after sodium citrate, greatly stimulated respiration. This is particularly interesting since mercury in untreated animals has little or no effect on respiration. Hence it is probable that the increased irritability of the respiratory center by citrate determined this new action of mercury. We have also observed that the effect of sodium citrate on blood pressure may be modified by the previous injection of adrenalin. Effective doses of citrate given 3 to 9 minutes after the injection of adrenalin either failed to produce any change in the circulation or caused a moderate rise of blood pressure. These observations suggested that the response to a substance may change when an organ is in a condition of increased sensitization, which has also been shown by other observers. It occurred to us that, as far as the circulation was concerned, we were probably dealing with increased sensitization also in the case of citrate and the allied salts when injected after pilocarpine. In view of the observations of Brodie and Dixon (11) on the vasomotor effect of pilocarpine it seems highly probable that the administration of sufficient amounts would produce increased sensitization of the vasomotor mechanism, sodium citrate under these conditions causing stimulation. As pointed out by us in a recent publication (2), its intravenous administration into animals with chest open either caused no change or was quickly followed by recovery and even a rise of blood pressure indicating rapid compensation of the depression produced on the heart, which clearly points to vasomotor stimulation. It may be expected, therefore, that when the vasomotor mechanism has been sensitized by pilocarpine, sodium citrate may readily cause stimulation and thus rapidly suppress or even overcompensate the cardiac depression which it produced. A case in point is the very brief duration of a fall of blood pressure preceding the rise which we occasionally observed when citrate or the other salt was injected after pilocarpine. We may turn once more to the effect produced by pilocarpine when it was injected after citrate and tartrate. The following results are suggestive in this connection. Burridge (12) found that the response of the isolated frog heart to calcium varied with the alkalinity of the perfusate. More recently Snyder and Campbell (13) made the very interesting observation in perfusing experiments of the vessels of the frog that the effect

of adrenalin changed with the hydrogen-ion concentration of the perfusing solution causing dilator action when above and constrictor effects when below that of the blood. As shown by Battelli and Stern (11) and later by Salant and Wise (15), sodium citrate is readily oxidized in the tissues with the formation of sodium carbonate. According to Neuberg and Saneyoshi (16) tartrates are likewise oxidized in the body, though not as completely as citrates. It may be assumed, therefore, that a change in the reaction of the blood thus occurs, which may affect the action of pilocarpine in a manner similar to that which Snyder and Campbell observed in the case of adrenalin. The numerous difficulties which were encountered in the attempts to explain the action of the intestine after pilocarpine and citrate or tartrate precluded the possibility of reaching definite conclusions as to the nature of the mechanism of the reversal changes. It may not be unprofitable, however, to call attention to the following manifestations of the behavior of the intestine, for on closer analysis they appear to be of some value in helping to understand the process which gave rise to this peculiar phenomenon. We observed that relaxation of the intestine occurred with greater frequency when citrate instead of tartrate was injected after pilocarpine and that oxalate as well as fluoride similarly administered affects the intestinal movements the same as in untreated animals. This suggested that the reversal phenomena of the intestine are in some way related to the oxidation of the salts. As stated above, oxalate alone caused in one experiment relaxation of the intestine with abolition of the rhythmic movements. The intestine, it was remarked, was in a state of firm tonic contraction when oxalate was injected, which recalls somewhat similar results obtained by Dixon (17) with strong lactic acid applied externally to the frog's stomach. He found that this substance caused different effects depending upon the condition of the organ. It is quite possible that a condition of fatigue is produced in the endings of the motor fibers of the vagus when the intestine is strongly contracted and overstimulated by pilocarpine or oxalate. When a fresh stimulus is given in this condition it probably acts upon the non-fatigued inhibitory fibers of the vagus. The mechanism may, therefore, be similar to that suggested for the action of sodium citrate and tartrate when injected after atropine.

The rôle which calcium plays in some of the changes produced by the different salts was discussed by Salant and Hecht (18) and later by Salant and Swanson (19). It was pointed out in these publications that the action of these salts could not be attributed to calcium precipitation

because the effect was not in proportion to the solubility of their calcium compounds. Analysis of the results obtained in the present investigation shows considerable variation in their behavior in the body, but the differences are in some cases quantitative rather than qualitative which signifies that disturbance of the calcium mechanism is probably a causal factor in their action, but it is evident that it is not the sole element concerned in the effects which they produce.

SUMMARY

1. The results of our investigations on the action of sodium citrate were corroborated. Intravenous injections depressed the circulation but stimulated respiration and intestinal movements, especially tonus.

2. Citrate was without effect or caused further relaxation of the intestine in atropinized animals. It also produced depression of the rhythmic movements. The effect of citrate on the intestine when atropine had been administered was attributed to stimulation of the end organs of the inhibitory fibers of the vagus. Citrate also depressed the intestine after pilocarpine alone had been injected. Fatigue due to overstimulation of the motor endings by pilocarpine and stimulation of the end organs of the inhibitory fibers by citrate were suggested as a probable cause.

3. Citrate after atropine and pilocarpine or pilocarpine alone, but not after atropine alone, produced a rise of blood pressure. Stimulation of adrenal activity by pilocarpine supported by increased sensitization of the sympathetic system was regarded as a possible explanation. But experiments with citrate and pilocarpine after removal of the adrenals were negative. Sensitization of the vasomotor system by pilocarpine was regarded as a plausible explanation of the reversal effects produced by citrate. Examples of sensitization of the vasomotor mechanism have been given in support of this explanation of reversal effect on circulation produced by citrate.

4. Intravenous injection of sodium tartrate produced effects similar to those of citrate but much larger doses were required to exert the same action as with citrate. Reversal phenomena observed when tartrate was injected after atropine and pilocarpine were similar to those produced by citrate under the same conditions, but some differences were noted.

5. Pilocarpine sometimes produced a considerable and persistent rise of blood pressure in animals that had received sufficient amounts

of citrate or tartrate. It also caused some depression of the intestine. Change of hydrogen-ion concentration is suggested as the cause.

6. Sodium oxalate in cats, but not in dogs, produced effects similar to those of citrate and tartrate. A rise, instead of a fall, of blood pressure was produced when oxalate was injected after large amounts of pilocarpine.

7. Sodium fluoride also produced a rise of blood pressure after pilocarpine but the circulation was depressed when sodium fluoride alone was injected in sufficient amounts. Respiration and intestine were stimulated but the effect was less than with corresponding amounts of the other salts.

8. The relation of the pharmacological action of citrate, tartrate, oxalate and fluoride to the effects which they produce on the calcium mechanism in the body was discussed above. The conclusion was reached that while disturbance of this mechanism may be an important element, it is not the sole factor in determining their behavior in the body.

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ON THE WEIGHT OF THE OVARIES IN THE ALBINO RAT DURING GESTATION AND NORMAL LACTATION: ALSO IN FEMALES DEPRIVED OF THEIR NEW-BORN LITTERS

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Received for publication February 18, 1923

For the weight of the ovaries on body weight in unmated albino rats, we have the data by Jackson (1), retabulated and charted by Hatai (2), and also published in *The Rat* (3).

These data apply to the ovaries in females which had never been mated, and it was thought desirable to obtain for comparison records of the weight of the ovaries during the cycles of gestation and lactation. The present paper deals therefore with the weights of the ovaries in relation to these cycles.

The work was done on albino rats, from The Wistar Institute colony, that are fairly uniform in their organ composition. Several conditions were imposed. All the rats used had previously cast one or more litters of at least four young. The times of mating and the times of the birth of the litters were accurately observed and recorded.

All rats showing at the post-mortem a middle ear infection, represented by fluid or pus in the bulla, which was often found, were excluded from the records. We shall return to this matter later.

The ovaries were removed and weighed separately in a closed vial. When the records were reviewed, the females which weighed less than 150 grams or in which the smaller ovary was *less than half the weight* of the larger, were excluded.

With material thus selected the weight of the ovaries was followed, usually at intervals of 2 days, during *a*, gestation; *b*, lactation (normal); and *c*, in females deprived of their new-born litters. For convenience this last series will be referred to as the "deprived" series.

[1] When Dr. J. M. Stotsenburg died on January 2, 1922, he left unfinished a study on the ovaries of the albino rat. As a slight tribute to him, my friend and colleague, I have prepared this work for publication, so that it may appear under his name.—H. H. D.

The general data for these three series are as follows:

a. Gestation. Observations were made from copulation to 21 days—12 periods—with at least two observations at each period. The total number of cases was 34. The mean standard body weight (on body length) was 206 grams. The standard body weights were in all cases determined from table 68 in Donaldson (3). The right ovary was heavier in 55.5 per cent of the cases and on the average represented 51.1 per cent of the total weight of the ovaries. The average number of fetuses (after the 10th day) was 9.5.

b. Lactation (normal). Observations were made from the birth of the litter to 28 days—15 periods. The total number of cases was 55. The mean standard body weight was 194 grams. The right ovary was heavier in 49.1 per cent of the cases and on the average had 48.6 per cent of the total weight of both ovaries. The average number of young was 8.5 per litter.

c. Females deprived of their new-born litters—the "deprived" series. Observations were made from the birth of the litter to 26 days—14 periods. The total number of cases was 32. The mean standard body weight was 197 grams. The right ovary was heavier in 34.4 per cent of the cases, but on the average had 48.1 per cent of the weight of both ovaries. The average number of young was 8.0 per litter.

These data show that the mean standard body weights are about the same (200 grams) in the three series and that there is no significant difference between the weights of the right and the left ovary—the average of the data for the three series being right ovary 49.2 per cent of the total weight, or practical equality. The average number of young in the three series is high, but similar. The somewhat higher value during gestation agrees with the general observations that the number of young born tends to be less than the number of discharged ova or fetuses (4), (5).

Method of determining the change in the weight of the ovaries. To reduce the observed weights so that the results of all the observations are comparable, the following plan was adopted: The observed weight of the ovaries was compared with the value for the ovaries (on body weight) in the unmated female from the standard table 70 (3), taken on the standard body weight. Using these weights, designated as those of the "ovulating" ovaries, as a basis, the percentage difference, plus or minus, of the observed value, was determined.

Then, to obtain uniformity in the presentation, the ovulating ovaries in all the series were assumed to weigh 49 mgm., and using this as a

basal value the percentage difference previously determined was applied to it and the resulting value taken for the tables and charts. In the tables this is entered as the "computed value."

TABLE 1

Showing the weight of both ovaries in the albino rat—from the beginning to the end of gestation

DAYS OF GESTATION	NUMBER OF CASES	AVERAGE BODY WEIGHT		AVERAGE WEIGHT OF OVARIES			AVERAGE NUMBER OF YOUNG
		Observed	Standard	Observed	Ovulating	Computed	
		grams	*grams*	*mgm.*	*mgm.*	*mgm.*	
0	6	186	185	91	48	92	
2	2	175	181	79	48	80	
4	3	195	191	73	49	73	
6	2	210	210	74	49	72	
8	2	223	220	83	50	82	
10	2	199	177	83	48	84	12.0
12	5	198	186	62	49	62	7.1
14	2	191	170	71	48	73	8.5
16	2	218	206	90	49	90	8.5
18	2	232	190	105	49	105	9.0
20	4	289	224	118	50	116	12.2
21	2	290	219	125	50	124	11.0

TABLE 2

Lactation (normal)

The weight of the ovaries during normal lactation

DAYS OF LACTATION	NUMBER OF CASES	AVERAGE BODY WEIGHT		AVERAGE WEIGHT OF OVARIES			AVERAGE NUMBER OF YOUNG
		Observed	Standard	Observed	Ovulating	Computed	
		grams	*grams*	*mgm.*	*mgm.*	*mgm*	
0	8	195	190	80	49	81	7.7
2	4	180	192	89	48	89	9.5
4	3	179	184	81	48	82	10.3
6	5	206	206	77	49	76	8.0
8	5	177	187	60	48	61	8.8
10	6	184	184	53	48	54	9.1
12	4	199	216	61	49	60	8.5
14	2	183	191	59	49	59	10.0
16	4	173	173	48	48	49	7.5
18	4	198	220	46	50	45	9.0
20	2	169	215	48	49	48	8.0
22	2	187	173	50	48	50	8.0
24	2	174	208	38	49	37	7.5
26	2	164	195	61	49	61	7.5
28	2	172	187	39	49	40	7.0

TABLE 3

Deprived of litter

The weight of the ovaries in females deprived of their new-born young

DAYS AFTER REMOVAL OF LITTER	NUMBER OF CASES	AVERAGE BODY WEIGHT		AVERAGE WEIGHT OF OVARIES			AVERAGE NUMBER OF YOUNG
		Observed	Standard	Observed	Ovulating	Computed	
		grams	*grams*	*mgm.*	*mgm.*	*mgm.*	
0	4	188	199	83	48	84	7.5
2	4	199	182	78	48	79	7.5
4	2	157	148	72	47	75	10.0
6	2	185	182	84	49	84	5.5
8	2	240	242	93	50	91	9.5
10	2	209	206	76	49	76	8.0
12	2	205	198	116	49	116	10.0
14	2	175	189	86	49	87	6.5
16	2	181	206	72	49	72	5.0
18	2	177	188	94	49	95	10.5
20	2	188	192	79	49	79	6.0
22	2	216	228	112	50	109	8.5
24	2	209	216	90	50	88	7.5
26	2	203	193	92	49	92	10.0

Explanation of the tables. In the tables, the standard body weight, rounded to grams, is that to be expected from the observed body length as given in table 68, (3). In the gestation series the observed body weight is entered without correction for the fetuses in the pregnant animals. The relation between the standard and observed body weight values will be considered in another place.

The weight of each ovary was taken separately to a tenth of a milligram. In the tables only the weights for the two ovaries combined are given, and these in turn have been rounded to milligrams. The method of obtaining the computed from the observed weight has already been stated. Inspection of the tables shows that in all the series the computed values for the weights of the ovaries differ but slightly, if at all, from the observed values. Despite the small change induced by computation, it has been thought worth while to give the full data. Finally, in each case, the average number of fetuses or young for each group is given, as this is an important datum when considering the weight of the ovaries.

By this procedure therefore a standard table was used as the point of departure and the final deviation was also always measured from a fixed base line. It may be said, in passing, that the values directly observed show, when plotted, much the same relations as do those more carefully computed.

In the tables 1 to 3 only the average computed values for each period are given, while to show the range, the individual computed values for each period have also been entered on the charts 1 to 3. When presented in this way the data stand as in tables 1 to 3 and charts 1 to 3.

Discussion of charts. The results obtained are most readily discussed by the aid of charts 1 to 3.

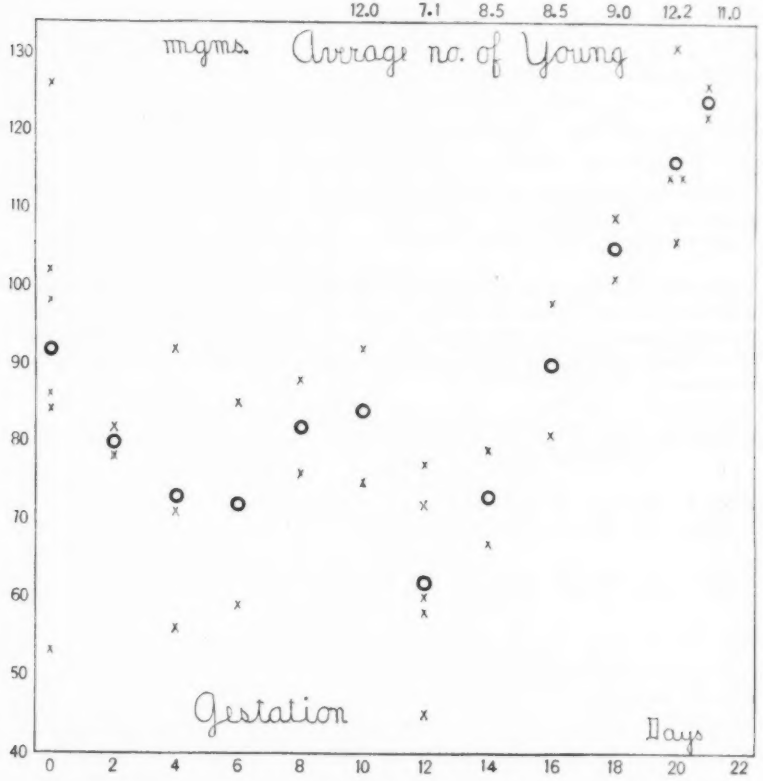

Chart 1. Based on table 1 and giving in milligrams the weight of both ovaries at twelve periods during gestation. The mean value for each period is indicated by a heavy circle—the individual values by a cross. Days entered below and the average number of fetuses above. The mean ovulating weight of the ovaries is 49 mgm.

Chart 1—Gestation. In this cycle the ovaries are heavy at the time of copulation, the weight then falls to about the 12th day, after which it rises, reaching a high value at the time of parturition. As will be seen later, the final high value in this instance is in part related to the large number of fetuses (corpora) present.

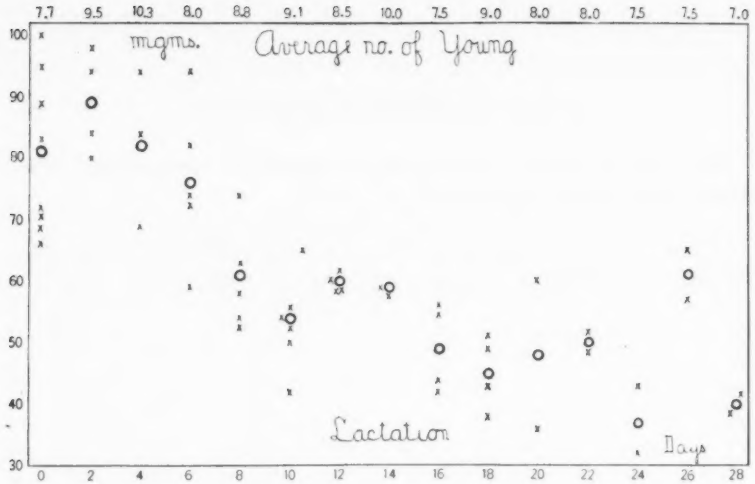

Chart 2. Based on table 2 and giving in milligrams the weight of both ovaries at fifteen intervals during lactation. The mean value for the period is indicated by a heavy circle. The individual values by a cross. Days entered below and the average number of young above. The mean ovulating weight of the ovaries is 49 mgm.

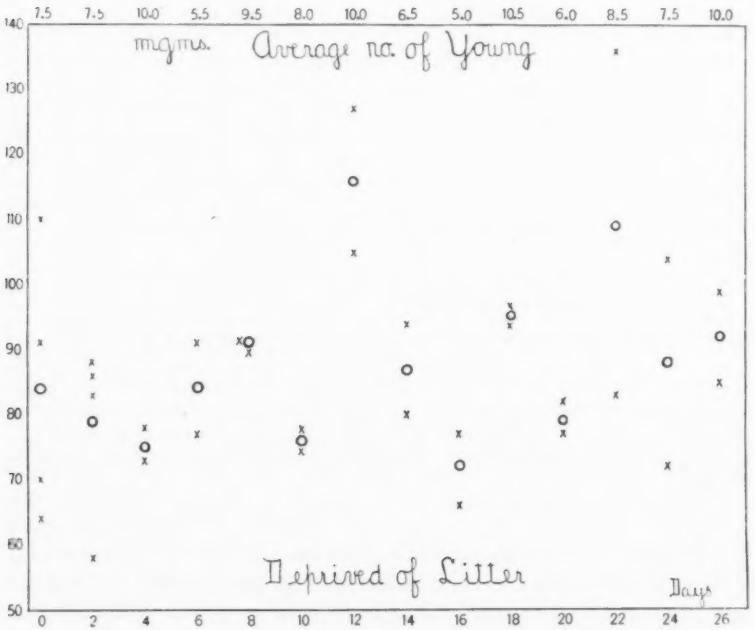

Chart 3. Based on table 3 and giving in milligrams the weight of both ovaries at fourteen intervals after removal of the litter at birth. The mean value for each period is indicated by a heavy circle—the individual values by a cross. Days entered below and the average number of young above.

The mean ovulating weight of the ovaries is 49 mgm.

Chart 2—Lactation (normal). Starting with a high value at the time of parturition the ovaries steadily decrease in weight, reaching approximately the ovulation value (49 mgm.) at the end of the lactation period at 22 days. The ovulation value is that given in table 70 (3) for unmated females.

Chart 3—"Deprived." The weight of the ovaries is of course high at parturition, at which time the rat is in a normal state. Then, removal of the litter brings about an abnormal condition. For the first 4 days there is a slight fall in weight, after which the weight rises slightly. The fall in the weight of the ovaries characteristic for normal lactation is therefore absent in this series. The mean value which is apparently most aberrant is that at 12 days. In this instance the number of young is large, and this in part accounts for the result.

TABLE 4

Showing the differences between the average body weights—observed and standard —at two intervals in each series

	BODY WEIGHT		PERCENTAGE DEVIATION OF OBSERVED
	Observed	Standard	
1. Gestation:			
0–12 days	196	191	+ 2.7
14–22 days	253	206	+22.2
2. Lactation:			
0–14 days	189	193	− 2.2
16–28 days	179	196	− 8.9
3. "Deprived."			
0–14 days	195	193	+ 1.0
16–26 days	196	204	− 4.0

Although the charts show that in all the series the weight of the ovaries at any period is highly variable, yet in the three series examined there are apparently characteristic values for the weights of the ovaries at successive periods. It is necessary therefore to consider on what general conditions these weights may depend, but before taking up that matter a few introductory statements should be made.

Touching the body weight. The group data on body weight, comprised in tables 1 to 3 yield, when averaged, the values which appear in table 4.

In the gestation series the excessive value of the body weights as observed after 14 days (+ 22.2 per cent) is plainly due in large measure to the weight of the developing fetuses (6), probably combined with some accumulation of fat.

In the normal lactation series the progressive decrease in the observed body weight is due probably to the loss of fat by the nursing mother (7).

In the "deprived" series there is some deviation of the observed from the standard, but we have no data on which to base an explanation. Since however the condition is abnormal, it is natural to expect a loss in body weight (fat) such as seems to have occurred in the last half of the series.

In general, then, satisfactory explanations can be given for the deviations of the observed from the standard body weights in all three series. It was because of these deviations that the standard, and not the observed body weight, was used for all computations.

Touching the weight of the ovaries. Inspection of ovaries in different stages of activity shows variations in the number and size of the follicles and corpora lutea. When the relation of the weight of the ovaries to

TABLE 5

Average number of fetuses or of young in the several series

	AVERAGE NUMBER OF FETUSES	AVERAGE NUMBER OF YOUNG
1. Gestation:		
10–22 days	9.5	
2. Lactation:		
0–28 days		8.5
3. "Deprived."		
0–26 days		8.0

follicle and corpora formation is recognized, it follows at once that the conditions which give rise to many of these structures in an ovary will cause a corresponding increase in weight (8). By contrast, an ovary in which they are few, will have a smaller weight. The number of corpora formed in any case is equivalent to or greater than the number of fetuses or young present, and for this reason the data on these latter have been summarized from our records.

In the gestating rat this enumeration was not made until the tenth day of gestation when the fetuses are clearly visible on dissection. When tabulated, these data appear as in table 5.

The size of the litters which occur in the lactation and "deprived" series is high, even when compared with data for second litters only (9). The average number of fetuses found in the gestation series is still higher, but in view of the prenatal mortality to which reference

has been already made, we infer that the number of living young cast would have been about that found in the other series. As judged by the number of young, therefore, the ovaries in these three series should have had approximately the same number of corpora formed in them, and in this respect should be comparable in the several series.

On the influence of the number of young on the weight of the ovaries. The full data (not given) contain records of the number of young or fetuses in each case in all three series. It is possible therefore to determine whether there is a correlation between the weight of the ovary and the number of young.

Working with the data which appear in charts 1 to 3 and using the average values for those above or below the mean—combined with our records for the number of young in each case, it appears that the ovary weights in each group are correlated with the number of young as follows:

	GROUPS WITH POSITIVE CORRELATION	TOTAL NUMBER OF GROUPS
In gestation series	7	8
In lactation series	12	15
In "deprived" series	9	14

Thus in 75 per cent of the groups a positive correlation is found.

Our data support therefore the view that there is a good correlation between the number of young and the weight of the ovaries. The effect on the ovaries is of course produced by the number of corpora, and the number of young serves merely as an indicator.

Following this idea and assuming that the deviation in the weight of the ovaries from the ovulation weight is due to the corpora—all of the ovary weights were computed on the basis of 8.5 young in the litter—thus eliminating the effect of differences in litter size.

The values thus calculated have not been entered in the tables or on the charts, but as they stand they distinctly smooth the record without however changing the general relations at successive periods.

Effect of repeated littering. There is still one other matter to be considered. In some cases the female had had more than one previous litter, and the effect of this on the weight of the ovaries is not known. The present data however shed some light on this question.

In the entire three series, comprising 123 cases, there are 14 in which the female had had more than one litter previous to breeding her for these series. These cases occur only in the lactation and "deprived"

series. It was something of a surprise to find that in the lactation series five of these cases showed ovaries with a weight below that of the period group to which they belonged, while three cases gave values that were above. In the deprived of litter series all six cases showed ovary weights below those for the corresponding period groups. Thus 11 out of the 14 cases showed low ovary weights.

A study of the detailed tables revealed something else, namely, that the number in the litter in these 11 cases giving a low ovary weight, was below that for the corresponding period group in eight instances and equal in three; while in the three cases with an ovary weight above that for the period group the litter number was equal to that of the group in one and greater in the remaining two.

From these data then there is no evidence for a progressive increase in the weight of the ovaries as the result of repeated littering. Moreover, the records show that in these cases under discussion the second litter was larger than the subsequent litters—thus agreeing with the relation pointed out by King (9).

It appears therefore that since the rats in this lot were bearing litters later than the second, and since these later litters tend to be smaller than the second litter, which is the one involved in all the other cases here examined, we should expect the ovaries from this lot to have a lower weight than that for the period group to which they belonged. This was the relation found. Aside from this effect, repeated littering does not appear to influence directly the weight of the ovaries.

Two pathological groups require a brief consideration: A. Rats with middle ear disease, represented by fluid or pus in the bulla. When this infection was first noted at autopsy, it was thought that the ovary weight was materially reduced by it, and hence the data for these animals were excluded. Sixty-six such cases came to autopsy. There are records for one period in the gestation series, 9 periods in the lactation series and 6 periods in the "deprived" series, but all these may be considered together. In each age period the weight of the ovaries in those having middle ear disease was contrasted with the weight in the corresponding normal group.

On the whole, the weight of the ovaries in the infected rats is somewhat less than in the normal. This deficiency is most marked in the "deprived" series. Within the 16 period groups the greater weight of the ovaries goes with the larger litter size in 11 groups, or 69 per cent. This is in agreement with what we find in the uninfected rats. The non-conforming groups are moreover all of one type; namely, cases in

which the ovaries from the infected rats weigh *less* than they should for the size of the litter. In no case of the infected rats is the relation disturbed by ovaries relatively *too heavy* for the size of the litter.

The data indicate consequently that middle ear infection is associated with a slightly lighter ovary in the infected animals. The difference is not large but is sufficient to justify the exclusion of these infected rats from the records.

B. The ovaries in "killer" females. A familiar character in rat colonies is the female that kills her young either at birth or at some later period. Different females appear to have characteristic times for this performance. While these studies were in progress it was possible to make post-mortems on 13 such "killers" from our own colony and to weigh their ovaries. Without going into the details it may be briefly stated that the weights of the ovaries were found similar to those given by the normal rats at corresponding periods, and as a consequence we do not associate with *"killer" females* any peculiarity in the ovary weights.

Some ovaries, representing selected periods in each of the three series, have been prepared for histological study, but have thus far been used only to collate in a general way the weights of the ovaries with the number and size of the follicles and corpora lutea. No histological analysis has been attempted.

The interpretation of the changes in the weights of the ovaries can be made therefore only in the most general terms, but owing to the recent publication by Long and Evans (10) our results may be collated with their extensive and valuable observations.

1. Gestation. The weight of the ovary tends to diminish during the first 12 days and then increases to the time of parturition. Apparently regression predominates during the first period and then the corpora of gestation increase to the end of the cycle. In the present instance the increase is exaggerated by the large size of the fetal litters at the later periods.

This relation of the ovary weights during gestation is in harmony with the observations by Long and Evans (10), who state (p. 70) that "during the last days of gestation the corpora lutea of gestation are alone present, the corpora of previous ovulations having been brought to atrophy and resorption," and p. 63, loc. cit. "after the tenth day of gestation continued slow growth of the corpora of gestation permits them to attain dimensions never found in the corpora of ovulation."

2. Lactation (normal). During lactation the weight of the ovaries decreases to the end of the normal lactation period. The ovarian

structures as a whole are evidently undergoing a decrease in size during this cycle and by the end of lactation the ovary has its "ovulation" weight. During lactation ovulation is suspended—no new corpora are formed save the corpora of ovulation.

In this connection Long and Evans (10, p. 68) point out that the corpora of gestation are shrinking while the corpora of lactation (those formed at the ovulation which occurs within 24 hours post-partum) are growing and reach their maximum from the 14th to the 20th day of suckling.

The mean values in chart 2 show a steady fall to about the 18th day. The combined events therefore appear to cause a well-marked loss in weight up to this time after which the weight tends to increase. This initial loss in weight for the first 18 days is rather more marked than we should expect from the history of the corpora as given by Long and Evans—but an adjustment of the two sets of results will require further study.

3. Females deprived of their new-born litters. The arrest of the mammary function, due to removal of the young, appears to prevent the reduction in the weight of the ovaries which occurs during normal lactation. The data suggest that there is some loss during the first 4 days, but after this the oestrus cycle is reëstablished and the ovaries tend to increase rather than to diminish in weight.

Long and Evans (10, p. 70) note that when the young are removed at birth the corpora of the preceding pregnancy tend to disappear less rapidly than during normal lactation—a state of affairs that would fit with the course of the values in chart 3.

GENERAL CONCLUSIONS

1. During the cycles of gestation and lactation in the albino rat: *A*, Characteristic changes in body weights occur. *B*. The ovaries undergo regular changes in weight between the beginning and the end of the several cycles. *C*. The ovaries are generally heavier than those in unmated females, save after the 14th day of lactation.

2. When the female is deprived of her *new-born* litter, the regular loss in the weight of the ovaries is curtailed and the ovaries tend to remain heavy—up to 30 days after casting the young.

3. These cyclic changes in the weight of the ovaries depend on changes in the number and size of the follicles and corpora lutea. The changes in both size and number are highly variable.

4. The number of corpora is related to the numerical size of ths litter—hence at any period in these cycles females with larger litters tend to have heavier ovaries. Emphasis is put on the fact that only values in animals in the same series and in the same age period can be thus compared.

5. Repeated littering does not influence directly the weight of the ovaries, but as litters, after the second, tend to become smaller, there appears a corresponding decrease in the weights of the ovaries of rats bearing later litters.

6. Middle ear disease, represented by fluid or pus in the bulla, may produce a slight lowering in the weight of the ovaries.

7. In "killer" females there is no modification of the weight of the ovaries to be associated with the peculiar behavior of these females.

8. Though the two ovaries of a given rat are rarely alike in weight, nevertheless in our gestation and lactation series, the right and left are excessive in nearly the same number of cases, while their average weights are similar. In the "deprived" series, although the weights of the right and left ovaries are nearly the same, the right ovary was heavier in only 34 per cent of the pairs.

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EFFECT OF DIGESTIVE ENZYMES ON PITUITARY EXTRACT ACTION

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Received for publication February 18, 1923

Since the discovery of the pharmacologic action of the extract of the posterior lobe of the hypophysis by Schäfer and Oliver in 1895, preparations of the gland have been used extensively in medicine, and their value in therapeutics, especially in obstetrical practice, is well established. In treatment it has been given largely for its stimulating effect on smooth muscle and the drug has been administered almost entirely intramuscularly.

Schäfer, the original discoverer, and his co-worker, Hering (1) were the first to investigate the possibility of giving extracts of the gland by mouth. These workers concluded from their investigations that peptic digestive fluid destroyed the pressor activity of extracts of the hypophysis, but left the diuretic action unaltered. Reducing agents and tryptic digestive fluid were found to have no effect on any of the active constituents of the extract. They also found that no noticeable change in blood pressure or diuresis was produced by introducing the drug directly into the stomach. They concluded from this that the extract is absorbed too slowly to produce the usual symptoms.

Dale (2) after investigating the same problem came to somewhat different conclusions. He found that digestion of pituitary substance with peptic extract of proved activity failed to alter, to any perceptible degree, the pressor or diuretic principles. He concluded that the peptic extract of Schäfer and Hering contained some antagonistic substance, or that their animal was for some reason unresponsive to the pressor effect. On the other hand Dale (2) found that every active preparation of trypsin reduced the diuretic and pressor qualities of pituitary extract to nil after twenty-four hours' digestion. Commercial trypsin gave the same results. He found that subsequent injections of pituitary substance caused an increased flow of urine and the usual rise of blood pressure.

Hamil (3) obtained evidence from his work on cats to confirm the view that pituitary extract administered by mouth causes the characteristic uterine contractions, and that absorption takes place from the stomach. It is more rapid when the stomach is full and actively digesting. This worker also states that the intestinal juices (pancreatic) rapidly destroy the active principles of the pituitary gland.

Dudley (4) showed that histamin and the uterine stimulant of pituitary extract are not identical and that the former is not present in detectable quantities in extracts of the gland. The reaction of the two substances to butyl alcohol, chloroform, alkalis and trypsin serves to differentiate them. This worker found the uterine stimulant of pituitary extract was almost completely destroyed by trypsin, (digested one-half hour) while histamin was unaffected by the latter when acted upon for this period of time. The authors made no observations upon the results which longer digestion might have revealed.

There is considerable clinical and experimental evidence to show that pituitary extract has, when administered by mouth, an appreciable effect on tonus of uterine muscle and also an antidiuretic effect. Donaldson (5) reports the results of the use of the dried tablets of pituitary extract by mouth in the treatment of uterine hemorrhage. It was used in fifty cases. The extract was given in 2 grain doses three times a day. These cases had all been previously examined for some mechanical cause for the hemorrhage and such conditions as fibroids, endometritis and carcinoma were excluded. In only four of these cases was no benefit reported from the use of pituitary extract and thirty-seven cases were greatly relieved by its use.

Morris and Weiss (6) report the successful use of pituitary extracts by mouth in a case of dyspituitarism.

Rees and Olmstead (7) used the dried extract by mouth in a case of diabetes insipidus and obtained a very definite decrease in the amount of urine passed and in the thirst accompanying the diuresis. Further clinical evidence of the value of pituitary extract when administered by mouth is not extensive.

In view of these clinical results and the fact that previous workers are not agreed as to the place and method of destruction of pituitary extract in its course through the intestinal tract (if it is destroyed) and if not destroyed where it is absorbed, we were led to conduct a series of investigations relative to the action of gastric, pancreatic and intestinal enzymes, on pituitary extract, including the above-mentioned problems. The work included the action of pituitary extract and of enzymes, and

of mixtures of these on uterine contractions (isolated strips); and the effect of these substances separately, and mixed, on blood pressure and uterine contractions of the living animal, with the uterus in situ.

Effect of digestive enzymes and pituitrin, and mixtures of these upon the contractions of isolated uterine strips: The fresh enzymes were either obtained directly from dogs, or prepared from the fresh tissues of dogs immediately after death. The tissues used were gastric and intestinal mucosa and fresh whole pancreatic gland.

The gastric mucosa extract was prepared by removing the gastric mucosa of the dog, which was then macerated and the tissue digested for 24 hours in 0.4 per cent HCl, at the end of which time the extract was strained and finally filtered. Its activity was tested by the biuret test.

The inactive extract of gastric mucosa was prepared by extraction with glycerol of the macerated gastric mucosa of the dog.

Extract of intestinal mucosa was prepared by digesting the macerated tissue in 0.9 per cent NaCl for 24 hours and the tissue shreds filtered off. The filtrate was preserved in 5 per cent toluol.

Pancreatic extract was prepared by macerating the ground-up gland, freed from fat, in 30 per cent alcohol for 24 hours, filtering at the end of this time and neutralizing the filtrate partially with KOH solution (5 per cent) and exactly with 0.5 per cent sodium carbonate solution.

Secretin was prepared by digesting the mucosa of the duodenum with 0.4 per cent HCl for 24 hours, after which the supernatant fluid was boiled and neutralized by sodium carbonate solution (0.5 per cent).

Pancreatic juice was obtained directly from the duct after stimulating the flow of the secretion by intravenous injections of secretin. Bile was obtained directly from the common bile duct.

Two proprietary preparations of enzymes were also used—Holadin, which contains the active principles of the pancreas, and peptenzyme, which contains the active gastric enzymes.

All pituitary extract used in this work was in the form of pituitrin O, P. D. & Co. Dilutions were made as indicated in the tables below.

Uterine strips were removed from virgin guinea pigs and these were suspended in oxygenated Ringer's solution kept at 37°C. The apparatus was arranged so that a small current of oxygen passed through the solution continuously. The uterine strip was suspended by means of a glass tube bent so as to allow the current of oxygen to flow in near the bottom of the glass container. This latter was made from the cylindrical part of a 100 cc. graduate, which itself was suspended in a copper

bottomed, galvanized iron tank. The tank contained water which was kept at 37°C. by heating a copper coil which was soldered into the bottom of the tank.

An opening in the bottom of the heater, which was closed by a cork through which a T-tube was run, allowed for introduction of oxygen and fresh solutions when needed.

TABLE 1

Effect of digestive enzymes in various preservatives on isolated strips of uterus of guinea pigs

SUBSTANCE ADDED TO CONTRACTION CHAMBER	EFFECT ON UTERUS			REMARKS
	Increased tone	Decreased	No effect	
1 cc. gastric M. ext.	Slight			
1 cc. gastric M ext. (glycerinated)		x		Results attributed to glycerine
Glycerine		x		
1 cc. gastric M. ext. (in 0.4 per cent HCl)			x	
			x	
1 cc. 0.4 per cent HCl	x			
1 cc. peptenzyme sol. (3 gm.)	x			
0.3 gm. peptenzyme			x	
1 cc. duodenal ext. (toluol)		x		Results attributed to toluol
1 cc. toluol		x		
1 cc. intestinal mucosa ext.	Slight			
2 cc. intestinal mucosa ext.				
1 cc. pancreatic ext. (activated)			x	
1 cc. pancreatic juice	Slight			
1 cc. pancreatic juice	x			
Holadin (2 gm.)	Slight			
1 cc. secretin sol.	Slight			
1 cc. pancreatic ext.			x	
1 cc. secretin			x	

The suspended uterine strip was attached by its free upper end to a heart lever and by it uterine contractions were recorded on a revolving kymograph.

The results obtained in this work are given in the accompanying tables 1 and 2.

As shown in table 1, all enzyme preparations except one when added to the fluid covering the uterine strips caused a slight increase of tonus.

The intestinal mucosa extract gave a fall in tonus except in one case where no fall was noted. We attributed this fall of tonus to the toluol used as a preservative, since the latter was found to cause the same fall of tonus as the extract.

The preparation, peptenzyme, seemed to give a more pronounced increase of tonus than the other enzyme mixtures. The rise of tone caused by the enzymes was not long sustained and at times no rise at all was obtained from addition of the enzyme alone.

The extracts prepared with glycerol gave a fall in tonus which was found to be due to the glycerol itself.

Secretin gave only a slight increase of tone.

On two occasions pancreatic extract activated with intestinal mucosa extract gave no change in the tonus.

In no experiment was the tissue permanently injured so that its tonicity and contractility were destroyed by any of the enzymes used.

In table 2 are shown the results obtained when pituitrin mixed with enzymes was added to the Ringer's solution in which the uterine strips were suspended.

Addition of 1 cc. of gastric juice activated by 0.4 per cent HCl mixed with 1 cc. of pituitrin O (1:10,000) to the muscle chamber resulted in increased tonus of the uterine muscle and results were directly comparable to those obtained when the same amount of pituitrin O was added alone. Digestion of the pituitrin with gastric mucosa extract for 2 hours failed to decrease the activity of the gland extract.

Similar results followed the addition of peptenzyme and pituitrin O to the muscle chamber, except that the former substance seemed to enhance the effect of pituitrin on uterine tone. In this work 0.3 gram tablets of peptenzyme were used mixed with 1 cc. pituitrin O (1:10,000). The same results were obtained with activated and non-activated peptenzyme mixed with pituitrin.

The action of human gastric juice on pituitrin was tested over several hours ($\frac{1}{2}$ to 2 hours). The results show a much greater increase of tone after 2 hours' digestion than from a half-hour digestion. This would seem to indicate that the action of pituitrin is merely delayed and not destroyed by gastric juice.

Duodenal extract of mucosa containing the enzymes of the succus entericus was mixed with pituitrin O (1:10,000) and the mixture digested 15 minutes at 37°C. No demonstrable decrease in the action of pituitrin thus treated could be recorded upon subsequent addition of the mixture to the muscle chamber.

Activated and unactivated preparations of the whole pancreas containing the pancreatic enzymes were tested in a similar manner. Pituitrin O (1:10,000) and undiluted pituitrin O were used, and mixtures of

TABLE 2

Effect of digestive enzymes on the action of pituitary extracts on isolated uterine strips from the guinea pig

The number of x's indicates the comparative change in tone

ENZYMES ADDED TO CONTRACTION CHAMBER	PITUITRIN ADDED	DIGESTION TIME	EFFECT ON UTERUS		
			Increased tone	Decreased tone	No effect
1 cc. gastric mucosa extract	1 cc. pit. O 1:10,000	5 min.	x		
Peptenzyme (activated)	1 cc. pit. O	5 min.	xx		
1 tab. peptenzyme	1 cc. pit. O 1:10,000	5 min.	x		
1 cc. peptenzyme	1 cc. pit. O 1:10,000	5 min.	x		
1 cc. intestinal mucosa ext.	1 cc. pit. O 1:10,000	5 min.	x		
1 cc. pancreatic extract	Pit. O 1:10,000	5 min.	x		
1 cc. pancreatic ext. (activated)	1 cc. pit. O digested 1 hr.	5 min.	x		
3 cc. pancreatic ext. (activated)	1 cc. pit O	5 min.	x		
0.2 gm. Holadin (activated)	1 cc. pit. O 1:10,000	5 min.	x		
Holadin 0.185 gm.	2 cc. 1:100,000	½ hr.	x		
Holadin 0.185 gm.	2 cc. 1:100,000	1 hr.	xx		
Holadin 0.185 gm.	2 cc. 1:100,000	1½ hr.	xxx		
Holadin 0.185 gm.	2 cc. 1:100,000	2 hr.	xxxx		
Holadin 0.185 gm.	½ cc. 1:100,000	2 hr.	xxxxx		
Human gastric juice 4 cc.	2 cc. 1:10,000	½ hr.	x		
Human gastric juice 4 cc.	2 cc. 1:10,000	1 hr.	xx		
Human gastric juice 4 cc.	2 cc. 1:10,000	1½ hr.	xxx		
Human gastric juice 4 cc.	2 cc. 1:10,000	2 hr.	xxxx		

these with the enzymes continued to cause increase of tone, in one instance after digestion for 1 hour with pancreatic extract at 37°C.

Holadin when mixed with pituitrin failed to decrease the action of the latter on uterine muscle.

Pituitrin digested by Holadin (a commercial preparation containing active pancreatic enzymes) for periods ranging from ½ to 2 hours showed some increase of tone at the end of the half-hour period, more after 1 hour and still greater tone increasing power at the end of 1½ hours' digestion, compared with that following ½ hour's digestion. This would seem to support the possibility that the pancreatic enzymes merely delay and do not destroy the effect of pituitrin on uterine muscle.

Addition of pituitrin O mixed with enzymes to the contraction chamber, following an initial dose of the hormone resulted in continued rise of tone. The following results were obtained in one series of experiments.

One cubic centimeter of pituitrin (1:10,000) caused an increase of tone. This was followed by addition of 1 cc. duodenal extract, 1 cc. pancreatic extract and 1 cc. gastric mucosa extract with pituitrin added to each in succession. The record shows a continued rise of tone after each addition of the mixture of enzymes and pituitrin.

Effect of pituitary extracts on blood pressure when injected directly into the lumen of the alimentary canal. Dogs were used as experimental animals. Injections were made into the lumen of the stomach, upper jejunum, ileum and cecum, 1 cc. of pituitrin O was injected into each viscus in the first series with no noticeable effect on blood pressure. In another series 2 cc. injections were made but in no case was there any change in the blood pressure.

It should be noted in this connection that the insertion of a hypodermic needle through the wall of the stomach or intestine will often give a rather pronounced rise in blood pressure, hence the injection should not be made until the effect from inserting the needle has had time to pass off.

Effect of pituitary extract when injected into lumen of alimentary canal, on blood pressure and uterine contractions. Dogs were used as experimental animals. The uterine contractions were recorded by the Barbour method.

It will be noted that in no case was there any effect on blood pressure.

Injection of pituitrin O into lumen of stomach gave an increased uterine tone when pylorus was unligated but on ligating the pylorus the injection did not affect the uterus.

Effect of intravenous injections of digestive enzymes on blood pressure. Dogs were used as experimental animals.

TABLE 3

Effect of pituitary extracts on blood pressure and uterine tone

Extract injected directly into the lumen of the alimentary canal

PITUITRIN O	WHERE INJECTED	EFFECT ON B. P.	EFFECT ON UTERUS
cc.			
1	Lower duodenum	None	Increased tone
1	Lower duodenum	None	Increased tone
1	Lower duodenum	None	Increased tone
1	Stomach	None	Increased tone
1	Stomach pylorus ligated	None	No effect

Gastric mucosa extract gave such a slight rise in blood pressure that it may be recorded as a questionable effect. On repeating this experiment we obtained either a drop or no effect. All other enzymes used gave a drop in blood pressure.

TABLE 4

Effect on blood pressure from intravenous injection of digestive enzymes

ENZYME	AMOUNT INJECTED	EFFECT ON B. P.
	cc.	
Gastric mucosa ext. in 0.4 per cent HCl	4	Very slight rise
Intestinal mucosa extract	4	Slight drop
Pancreatic ext. (alcoholic) activated	4	Marked drop
Pancreatic juice (dog) activated	1	Marked drop
Bile, dog	1	Marked drop

Effect of digestive enzymes on blood pressure and uterine contractions. Dogs were used as experimental animals. Uterine contractions were recorded by the Barbour method. The enzyme preparations were given intravenously.

TABLE 5

Effect of intravenous injection of digestive enzymes on blood pressure and uterine contractions

ENZYME PREPARATIONS	AMOUNT INJECTED	EFFECT ON B. P.	ON UTERUS
	cc.		
Peptenzyme	2	Drop	None
Gastric mucosa, HCl ext.	2	Drop	None
Pancreatic extract	2	Drop	None
Holadin	2	Drop	None
Intestinal mucosa ext.	2	Drop	None

In every case the intravenous injection of enzyme preparations gave a drop in blood pressure. The most pronounced drop was obtained with intestinal mucosa extract.

None of the enzymes used showed any effect on uterine contractions.

Effect of pituitary extracts plus digestive enzymes on blood pressure and respiration. The pituitary extract was mixed with the digestive enzymes and the mixture was allowed to stand at body temperature for various lengths of time. The mixture was injected intravenously. Dogs were used as experimental animals.

TABLE 6

Effect of digestive enzymes on the pressor action of pituitary extract

ENZYME	PITUITRIN O	DIGESTION TIME	BLOOD PRESSURE	RESPIRATION
	cc.	*minutes*		
Pancreatic extract, 1 cc.....	0.5	30	Drop then rise	No effect
Pancreatic extract, 1 cc.....	0.5	30	Drop then rise	No effect
Intestinal mucosa ext., 1 cc...	0.5	3	Prolonged rise	No effect
Pancreatic ext. activ., 1 cc....	0.5	1	Drop then rise	No effect
Gastric juice 1 cc.....	0.5	1	Rise	

The pressor action of the pituitary extract is apparently not destroyed by the digestive enzymes used. In a few cases the drop produced by enzymes shows up first, followed in a very short time by the pressor action of the pituitary extract.

SUMMARY AND CONCLUSION

The above results may be interpreted as indicating that the uterine tone-raising action of pituitary extracts is not destroyed by the gastric and intestinal enzymes used in this investigation.

With the direct application of pituitary extracts and enzymes to the uterine muscle this was found to be true but that activity of pituitary extracts is not changed at all in the alimentary canal can not be conclusively stated from this series of experiments.

In our opinion certain pharmacologic properties of pituitary extracts may be changed or destroyed by digestive enzymes without altering other properties.

According to the investigations of several workers there are two and possibly three distinct constituents in the hypophyseal secretion (posterior lobe) which have different chemical constitutions, and are acted upon differently by various chemical reagents. It seems reasonable

from analogy to other chemical reactions that one constitutent may be changed or destroyed while another may remain unchanged by digestive enzymes.

It has been suggested to us that bacterial action in the intestine may be responsible for a change in the active properties of pituitary extracts. This does not seem to have been a factor in our work since the time element is probably too short for bacterial action and also because bacteria are generally considered to be few in number in upper portion of the small intestine.

On the isolated uterus of the guinea pig digestive enzymes alone may, in some cases, give an increase in tone but in these cases the increase is slight.

When pituitary extract was added to the enzyme there was in every case an increase in uterine tone which was practically equal to that obtained by pituitary extract alone in the same dilution.

This action is apparently not affected by the time (up to 2 hours) that the extract is allowed to digest with the enzyme.

With the uterus in situ in dogs the intravenous injection of digestive enzymes alone gave a uniform drop in blood pressure but in no case was there any effect on uterine tone.

When pituitary extract was added to the enzymes and allowed to digest for various periods of time the intravenous injection of the mixture always gave a rise in blood pressure.

The respiration was unaffected by intravenous injection of enzymes or pituitrin or of mixtures of these into the femoral veins of dogs. Results obtained conflict with those obtained by Fuehner (8) on rabbits, as the latter secured marked effect on respiration in these animals from intravenous pituitrin administration.

Direct injection of pituitrin into the alimentary canal resulted in increased uterine tone, the drug being absorbed in 50 seconds to 2 minutes, the effect lasting 10 to 15 minutes. Injection into other parts of the intestinal tract were without effect on uterine tone except when injection was made into the stomach with pylorus left open, when the same result was obtained as when the drug was injected into the duodenum. This shows that the drug is absorbed in a very limited area of the intestinal tract and absorption is rapid. Direct injection into the intestine resulted in no demonstrable effect on blood pressure. This effect needs further explanation but is probably not due to the destruction of the pressor principle of pituitary substance by the digestive enzymes, since this was found to be unaltered by the action of the enzymes.

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THE BLOOD ELEMENTS IN COMPLEMENT DEFICIENT GUINEA PIGS

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Received for publication February 20, 1923

A strain of guinea pigs deficient in complement was developed at the Vermont Agricultural Experiment Station (1). This is the only group of such animals known, a fact which makes them of considerable scientific interest. In that laboratory it was observed that complement deficient guinea pigs were considerably less resistant to disease and more susceptible to changes in temperature than normal guinea pigs; this was particularly noted when the temperature dropped to zero.

This lowered resistance suggested to us that other factors besides complement might be lacking in the blood of these animals. We have therefore determined the number of red and white corpuscles and the amount of complement and catalase in the blood. For these experiments we used 12 complement deficient guinea pigs and an equal number of controls, all of which had been kept under the same experimental conditions. Later 10 more controls were tested in the same manner.

Methods. The experimental procedure was as follows: a guinea pig was quickly etherized and about 3 cc. of blood drawn aseptically from the right auricle by means of a hypodermic syringe. The red and white corpuscles of this blood were counted under the microscope in the usual manner. A portion of the blood was defibrinated and 0.5 cc. added to 100 cc. of hydrogen peroxide, which had previously been diluted one-half with distilled water. The oxygen given off from this mixture served as a measure of the catalytic action of the blood.

A part of the blood which had previously been defibrinated was divided into six parts and 0.08 and 0.01 cc. of a 20 per cent solution and 0.04 and 0.3 cc. of undiluted serum respectively were titrated against 0.5 cc. of a 1 per cent solution of sheep erythrocytes and two units of amboceptor in each tube. As a check on the hemolysis no serum was put in one test tube and the corpuscles in another tube were not sensitized. Similar experiments were made to test the sera of the normal guinea pigs.

Complement test. No hemolysis took place in 43 tests when the complement deficient sera were tested, while in 5 tests only a very small amount occurred as shown in table 1. Complete hemolysis took place in each of the four tubes containing blood of controls.

TABLE 1

Test of the sera of complement deficient guinea pigs; 0.5 cc. of 1 per cent washed sheep erythrocytes and two units of amboceptor in each test tube

GUINEA PIG NUMBER	DATE	DEGREE OF HEMOLYSIS CAUSED BY THE SERA OF COMPLEMENT DEFICIENT GUINEA PIGS					
		Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
		0.08*	0.1*	0.04	0.3	0	0.3†
3	May 13	0	0	x	xx	0	0
6	May 13	0	0	0	xx	0	0
7	May 13	0	0	0	0	0	0
11	May 13	0	0	0	0	0	0
12	May 13	0	0	0	0	0	0
Control A		xxxx	xxxx	xxxx	xxxx	0	0
9	May 16	0	0	0	x	0	0
4	May 16	0	0	0	x	0	0
14	May 16	0	0	0	0	0	0
8	May 16	0	0	0	0	0	0
13	May 16	0	0	0	0	0	0
5	May 16	0	0	0	0	0	0
Control D		xxxx	xxxx	xxxx	xxxx	0	0

* Twenty per cent solution of sera in normal saline. The other amounts of sera are undiluted.

† Corpuscles not sensitized in these tubes.

A = Control guinea pig.

D = Control guinea pig.

xxxx, Complete hemolysis.

xx, Some hemolysis.

x, Very slight hemolysis.

0, No hemolysis.

Red and white blood corpuscles. In the complement deficient animals the red blood corpuscles as shown in table 2 varied between 4,800,000 and 8,200,000 with an average of 6,401,167 per cubic millimeter in the first test, and between 4,080,000 and 8,150,000 with an average of 6,718,000 in the second test.

In the control animals the number of red blood corpuscles ranged from 7,068,000 to 11,100,000 with an average of 8,667,667 in the first test and from 7,500,000 to 12,400,000 with an average of 8,742,100 in the second

TABLE 2

Complement deficient guinea pigs

NUMBER	FIRST TEST				SECOND TEST			
	Date	Sex	Number of red blood corpuscles per cu mm. of blood	Catalase in 0.5 cc. defibrinated blood	Date	Red blood corpuscles per cu mm. of blood	White blood corpuscles per cu mm. of blood	Catalase in 0.5 cc. defibrinated blood
7	April 26	Female	6,000,000	136.5 136.0 } 136.3	May 13	7,000,000	11,000	98.0
5	April 26	Female	5,200,000	146.5 152.5 } 149.5	May 16	5,500,000	17,000	108.5
11	April 26	Male	6,800,000	150.5 156.0 } 153.3	May 13	7,050,000	18,500	105.0
14	April 26	Male	5,600,000	144.0 150.5 } 147.3	May 16	5,900,000	19,000	103.0
9	April 27	Female	6,664,000	133.0 134.0 } 133.5	May 16	8,150,000	18,000	107.0
12	April 27	Female	4,800,000	112.0 116.0 } 114*				
13	April 27	Male	6,100,000	111.5 120.0 } 115	May 16	7,550,000	12,000	127.0
1	April 28	Female	6,000,000	143.0 149.0 } 146*				
6	April 28	Male	7,500,000	113.0 123.0 } 118	May 13	7,200,000	14,000	107.0
3	April 28	Male	7,200,000	126.0 125.0 } 125.5	May 13	8,000,000	15,000	103.0
8	April 28	Male	8,200,000	141.0 145.0 } 143	May 16	4,080,000	16,000	129.0
4	April 28	Female	6,750,000	88.0	May 16	6,750,000	19,000	88.0
Average			6,401,167	130.7		6,718,000	15,950	107.55

* Numbers 12 and 1 died after operation.

TABLE 3

Control guinea pigs

NUMBER	FIRST TEST				SECOND TEST			
	Date	Sex	Number of red blood corpuscles per cu. mm. of blood	Catalase in 0.5 cc. defibrinated blood	Date	Number of red corpuscles per cu. mm. of blood	Number of white blood corpuscles per cu. mm. of blood	Catalase in 0.5 cc. defibrinated blood
A	May 6	Male	7,068,000	330.0, 331.0 } 330.5	May 18	9,750,000	20,000	287
B	May 6	Male	10,400,000	302.5, 306.5 } 304.5*				
D	May 6	Male	11,100,000	306.5, 306.5 } 306.5	May 18	12,400,000	14,500	283
F	May 8	Male	8,000,000	304.0	May 18	8,320,000	16,500	314
G	May 8	Male	8,800,000	338.0, 334.0 } 336.0	May 20	7,800,000	10,500	274
H	May 11	Male	8,000,000	290.0, 282.0 } 285.0	May 18	7,500,000	9,900	280
C	May 6	Male	9,200,000	140.0, 150.0 } 145.0	May 18	8,600,000	17,500	126
E	May 8	Male	10,400,000	139.5, 141.0 } 140.3*				
I	May 11	Male	8,000,000	137.0, 136.0 } 136.5	May 18	7,700,000	7,500	113
J	May 11	Male	8,000,000	128.0	May 20	8,051,000	11,500	116
K	May 11	Male	7,444,000	153.0, 145.0 } 149.0	May 20	7,850,000	7,500	110
L	May 11	Male	7,600,000	147.0, 150.0 } 148.5	May 20	9,450,000	13,500	120
Average.....			8,667,667	226.2		8,742,100	12,890	202.3

* Died after operation.

test as shown in table 3. A second lot of 10 normal guinea pigs that had been kept out of doors showed an even higher red blood corpuscle count, ranging from 7,150,000 to 11,750,000 and averaging 9,815,000.

Thus the complement deficient guinea pigs were found to average from 37 to 50 per cent fewer red blood corpuscles than normal animals. This decreased number of erythrocytes should lessen the oxygen carrying power of the blood and this in turn would decrease the available oxygen for the tissues, which might account in part for the lowered resistance of these animals to zero temperature.

As to the number of white blood corpuscles they ranged in the complement deficient guinea pigs between 11,000 and 19,000 with an average of 15,950 per cubic millimeter of blood. In the control animals these counts varied from 7,500 to 20,000 with an average of 12,890. The guinea pigs that had been kept out of doors showed the lowest average number of white blood corpuscles of all, since they ranged from 5,000 to 14,000 with an average of 8,600.

The complement deficient animals averaged from 19 to 42 per cent more white blood corpuscles than did the control animals. This increased number may be a protective device on the part of the organism to make up for the lack of complement as a defense against the invasion of micro-organisms.

Catalase. The amount of oxygen given off from 0.5 cc. of defibrinated blood of the complement deficient guinea pigs ranged between 88 and 153.3 cc. with an average of 130.7 cc. in the first test, and between 88 and 129 with an average of 107.6 cc. in the second test. The hot weather occurring during the second test probably accounts in part for the lowered catalase (2).

Upon testing the controls we found that they fell into two distinct groups of six each, one with a low and the other with a high catalase content, as shown in table 3. The former group compared closely in this respect with the complement deficient animals for the amount of catalase ranged from 128 to 149 cc. with an average of 141.2 cc. in the first test and from 110 to 126 cc. with an average of 117 for the second test. In the other group the catalase averaged more than twice as high as in the low group or the complement deficient guinea pigs, for it varied from 285 to 336 with an average of 311.1 cc. in the first test and from 274 to 314 with an average of 287.6 in the second test.

The ten other normal guinea pigs were also found to fall into two groups of five each, one with a low and the other with a high catalase content. The amount of oxygen given off from the blood of the former

group ranged from 119 to 142 with an average of 127 cc. and in the latter from 253 to 342 with an average of 300 cc.

This fifty-fifty ratio in two different sets of normal guinea pigs suggests that high and low catalase may be Mendelian characters. Complement deficiency in these animals has been found to be inherited according to Mendel's law (1).

Upon comparing the blood counts of these high and low catalase groups of normal guinea pigs we found no consistent relationship between these different factors. In the first lot of normal animals the number of red blood corpuscles was slightly higher in the high catalase group than in the low, but in the second lot of controls the low catalase group showed more erythrocytes than the high. As to white blood corpuscles, in the the second lot of controls the low catalase group averaged more than the high, but in the first lot the contrary was true.

Unfortunately all of our complement deficient guinea pigs were lost through disease, so further experiments had to be abandoned.

SUMMARY

1. A relation between the number of red blood corpuscles and lack of complement was found in our animals. The complement deficient guinea pigs averaged from 37 to 50 per cent fewer red blood corpuscles per cubic millimeter of blood than did the normal animals. This decreased number should lessen the oxygen carrying power of the blood, and this in turn would decrease the available oxygen for the tissues and may account in part for the lowered resistance of these animals to zero temperature.

2. The average number of white blood corpuscles was 19 to 42 per cent higher in the complement deficient guinea pigs than in the controls. This is an indication of a protective device on the part of the organism to make up for the lack of complement as a defense against the invasion of foreign organisms.

3. No consistent relationship was found between the number of white and red blood corpuscles and the amount of catalase in the blood.

4. Two sets of normal guinea pigs fell into two groups as regards the catalase content of the blood; in half of the animals of each set the catalase was low, while in the other half it was more than twice as high. This fifty-fifty ratio in two different sets of normal guinea pigs suggests the possibility that high and low catalase may be Mendelian characters.

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THE RESPIRATORY EXCHANGE AND ALVEOLAR AIR CHANGES IN MAN AT HIGH ALTITUDES

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Received for publication February 24, 1923

Along with other data accumulated in a series of expeditions to the summit of Pike's Peak during the years 1911 to 1917, a number of respiratory studies were made that have not as yet been published. Among these are observations on the respiratory exchange and alveolar air. The respiratory exchanges of carbon dioxide and oxygen were followed in two expeditions and the changes in the alveolar air in four expeditions. Among the subjects were men in various degrees of physical fitness, men who ascended the mountain passively in a railway car, and others who climbed up afoot. The influence exerted by these conditions upon the alveolar air changes, and incidentally the problem of mountain sickness will be considered.

THE TOTAL RESPIRATORY EXCHANGE. Our gaseous metabolism observations were made on five men. In one expedition three of these subjects were studied. In the first experiment the subject sat at rest upon a bicycle in as relaxed a condition as possible with feet supported upon a platform; in the second he did a constant but moderate amount of muscular work upon a bicycle so mounted in a supporting frame that it was constantly worked against a slight, but not measured, resistance. The amount of work was made uniform by always requiring the subject to pedal at the same rate, 90 revolutions per minute. During another expedition a third set of experiments was made with three subjects, one of whom also served in the other studies. In this series the subject walked at the rate of 4 miles an hour over a course measured out on the floors of rooms adjacent to the laboratories of both altitudes.

The experiments were conducted indoors in order to eliminate any influence that environmental temperature might have on metabolism. The laboratory on Pike's Peak was heated by a coal stove to a temperature that ranged between 66° and 72°F.

The respiratory exchange was measured by the Douglas bag method (3) in which the expired air was collected during an exact interval determined with a stop-watch and the volume of air collected then accurately measured with a wet gas-meter. In our experiments the resting collections were made for a period of 4 minutes after the subject had been quiet for about half an hour and had been sitting at rest on the bicycle for 2 minutes. The collections of air, taken 2 minutes after beginning work on the bicycle, were continued for 2 minutes. The collections during walking were always made for 1 minute, between the 5th and 6th minutes.

The purpose of the experiments was to obtain information on the total respiratory exchange under the conditions of normal daily life. With the variety of experiments we were conducting while on Pike's Peak it was essential that each man keep as fit as possible and that he be ready to serve not only as a subject of experimentation but also as an observer or analyst. Hence the question arose as to the feasibility of conducting the experiments on men living the normal life of the laboratory worker without the discomfort attended with abstinence from food. Obviously the ingestion of food could not be disregarded. However, inasmuch as Benedict and Murschhauser (2) had published their results in which they had compared the metabolism of men before taking breakfast, after a light morning meal, and after a heavy noon meal; and had found that after the light morning meal the metabolism was 1.43 calories and after a heavy noon meal only 1.45 calories, and that the basal metabolism under these two conditions was increased approximately 16 per cent, it was decided to allow our subjects to eat the usual meals. The gaseous metabolism experiments were, as a rule, conducted with each subject in as nearly as possible the same post-absorptive condition.

Because it had been suggested that the discrepancies observed in data of Alpine researches might be explained on the assumption that some of the observations were made on subjects in the process of acclimatization while others were made only after acclimatization, it seemed desirable to make daily determination of the respiratory exchange throughout the entire period of the acclimatization.

The former studies of the gaseous metabolism of high altitudes have been taken up by three different methods; first, in pneumatic chambers; second, during balloon ascensions; third, in mountain ascents. Zuntz (20), working with a low barometric pressure of 448 mm. Hg in a pneumatic chamber, found that both at rest and during muscular exercise the exchange of carbon dioxide and oxygen was unchanged when

compared with the normal. Later Durig and Zuntz (5) in a sojourn of 2½ weeks on Monte Rosa at an altitude of 15,000 feet, barometric pressure 443 mm., found that the resting metabolism increased as much as 15 per cent. It was maintained that this increase was not caused by cold, since it was present when the individual was in a warm bed. During this expedition they found that an altitude of 10,000 feet did not influence the gaseous exchange; and later on the peak of Teneriffe (6), at an altitude of about 10,500 feet, the same negative results were obtained. In an expedition to Capanna Margherita on Monte Rosa in 1906, Durig (7) obtained in six men increases that ranged between 9.9 and 45.5 per cent in the amount of oxygen absorbed. Fuchs and Deimter (9), in 1909 on the same peak, obtained an increase in the production of carbon dioxide and absorption of oxygen that was disproportionately greater during standing than while reclining at rest.

Viali (19), at the Mosso Scientific Institute on Monte Rosa, has recently reported for two men a higher exchange at the high than the low altitude. In one subject the output of carbon dioxide was about 32 per cent and the intake of oxygen about 25 per cent above the normal during the first five days of the sojourn, while these almost returned to normal during the five succeeding days. In the second subject the gaseous exchange was not increased as much during the early days; but instead showed, throughout the stay, a maintained increment of about 11 per cent. The respiratory quotient was increased at the high altitude.

During the Anglo-American Expedition to Pike's Peak (4) in 1911, the average respiratory exchange of Douglas at rest in bed was almost the same as at sea level; however, the exchange on Pike's Peak was slightly greater. There were 248 cc. of oxygen and 206 cc. of carbon dioxide on Pike's Peak as opposed to 237 cc. of oxygen and 197 cc. of carbon dioxide in Oxford. During standing the exchange of these gases was slightly greater on Pike's Peak; but during walking at various speeds, up to 5 miles an hour, out of doors on Pike's Peak and on a grass track at Oxford, the curves showing the average absorption of oxygen were practically identical and could be exactly superimposed. This indicated that the gaseous metabolism during muscular exercise was unchanged with altitude.

Hasselbalch and Lindhard (13) each spent a number of days in a pneumatic chamber at atmospheric pressures that ranged from 589 to 448 mm. Hg with no apparent change in the exchange in carbon dioxide and oxygen.

The resting metabolism. Our own results indicate that there are individual differences that may be manifest in the metabolism at high altitudes and that these are most in evidence during muscular work.

TABLE 1

Respiratory exchange, sitting at rest on a bicycle

PLACE	DATE	CO_2	O_2	VOLUME OF AIR PER MINUTE AT PREVAILING BAROMETER	VOLUME OF AIR AT 0° AND 760 MM.	CO_2 EXHALED PER MINUTE	O_2 ABSORBED PER MINUTE	R.Q.
Cheley								
	1916	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
Colo. Springs	5, 19	4.12	16.33	8,360	6,195	253	293	0.863
Colo. Springs	6, 1	4.15	16.68	8,682	6,402	263	275	0.956
Colo. Springs	6, 2	3.99	17.00	7,870	5,867	232	231	1.003
Colo. Springs	6, 11	4.04	15.65	7,230	5,520	221	310	0.713
Average		4.08	16.42	8,036	5,994	242	280	0.884
Pike's Peak	6, 16	6.13	14.01	6,550	3,672	224	262	0.855
Pike's Peak	6, 17	6.14	14.56	8,070	4,498	274	290	0.945
Pike's Peak	6, 18	5.61	14.88	8,532	4,723	263	291	0.904
Pike's Peak	6, 19	5.72	14.51	8,682	4,684	266	310	0.858
Pike's Peak	6, 20	4.97	15.27	7,915	4,319	213	252	0.845
Pike's Peak	6, 21	5.82	14.71	7,770	4,229	244	268	0.910
Pike's Peak	6, 22	5.47	14.79	8,922	4,899	266	310	0.858
Average		5.94	14.68	8,063	4.432	250	283	0.882
Ross								
Colo. Springs	5, 19	3.97	16.67	9,522	7,080	278	300	0.903
Colo. Springs	6, 1	3.65	17.01	8,795	6,494	234	260	0.900
Colo. Springs	6, 9	3.61	17.07	8.682	6,523	233	257	0.907
Colo. Springs	6, 11	3.70	16.94	8,125	6,053	222	247	0.898
Average		3.73	16.92	8,781	6,538	242	268	0.902
Pike's Peak	6, 17	4.96	15.91	9,215	5,157	254	260	0.977
Pike's Peak	6, 18	4.50	16.32	8,882	4,917	219	229	0.956
Pike's Peak	6, 19	4.97	16.05	11,542	6,248	308	304	1.013
Pike's Peak	6, 20	4.09	16.71	10,867	5,872	238	249	0.956
Pike's Peak	6, 21	4.09	16.48	11,615	6,324	256	288	0.889
Pike's Peak	6, 23	4.09	16.40	12,170	6,672	269	301	0.894
Pike's Peak	6, 24	4.40	16.50	11,235	6,129	257	273	0.941
Average		4.44	16.34	10,789	5,903	259	272	0.947

TABLE 1—*Concluded*

PLACE	DATE	CO_2	O_2	VOLUME OF AIR PER MINUTE AT PREVAILING BAROMETER	VOLUME OF AIR AT 0° AND 760 MM.	CO_2 EXHALED PER MINUTE	O_2 ABSORBED PER MINUTE	R.Q.
Schneider								
	1916	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
Colo. Springs	5, 25	3.14	17.79	7,850	5,800	180	183	0.984
Colo. Springs	6, 2	2.99	17.53	7,715	5,755	170	202	0.842
Colo. Springs	6, 9	4.06	16.77	6,985	5,231	210	220	0.955
Average		3.40	17.36	7,517	5,595	187	202	0.927
Pike's Peak	6, 17	4.79	15.76	8,258	4,620	219	244	0.898
Pike's Peak	6, 18	4.75	15.72	8,477	4,632	218	247	0.883
Pike's Peak	6, 19	3.81	16.75	7,640	4,154	157	178	0.882
Pike's Peak	6, 20	4.12	16.42	8,022	4,374	178	202	0.881
Pike's Peak	6, 21	4.08	16.52	8,390	4,544	184	205	0.898
Pike's Peak	6, 22	4.33	16.34	8,520	4,620	198	216	0.917
Pike's Peak	6, 23	3.91	16.81	8,167	4,496	174	188	0.926
Pike's Peak	6, 24	4.74	15.82	8,747	4,786	225	250	0.900
Average		4.32	16.27	8,278	4,528	194	216	0.898

The data for our subjects sitting at rest on a bicycle are summarized in table 1. The average respiratory exchange during rest was for Cheley at 6,000 feet, carbon dioxide 242 cc., oxygen 280 cc., and at 14,110 feet, carbon dioxide 250 cc., oxygen 283 cc.; for Ross at 6,000 feet, carbon dioxide 242 cc., oxygen 268 cc., and at 14,110 feet, carbon dioxide 259 cc., oxygen 272 cc.; and for Schneider at 6,000 feet, carbon dioxide 187 cc., oxygen 202 cc., and at 14,110 feet, carbon dioxide 194 cc., oxygen 216 cc. During the sojourn on Pike's Peak each man had, therefore, on the average, a slightly greater output of carbon dioxide than in Colorado Springs. Cheley averaged an increase of 3.2 per cent, Schneider 3.7 per cent, and Ross 2.9 per cent, if the result for June 19th be eliminated from his data. These differences are too small to be significant. The increase in oxygen consumption averaged still less in Cheley and Ross (1.1 and 1.5 per cent respectively) but was somewhat more above normal in Schneider. With Schneider the output of carbon dioxide and consumption of oxygen were especially high during the first two days and the last day of his sojourn on the mountain, which accounts for the average increase of 6.9 per cent in his oxygen consumption. During the first two days this increase averaged 14.2 per cent for the carbon dioxide and 17.6 per cent for the oxygen.

The ranges for the output of carbon dioxide and consumption of oxygen for these men at the two altitudes also indicate that no clear-cut altitude difference in metabolism was present. The carbon dioxide

TABLE 2

Respiratory exchange, working on a bicycle

PLACE	DATE	CO_2	O_2	VOLUME OF AIR PER MINUTE AT PREVAILING BAROMETER	VOLUME OF AIR AT 0° AND 760 MM.	CO_2 EXHALED PER MINUTE	O_2 ABSORBED PER MINUTE	R.Q.
Cheley								
	1916	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
Colo. Springs	6, 1	4.91	16.09	23,517	17,320	843	837	1.01.
Colo. Springs	6, 2	4.63	16.75	24,167	18,000	826	700	1.18
Colo. Springs	6, 8	5.12	16.22	21,527	16,279	827	750	1.10
Colo. Springs	6, 11	5.45	15.35	22,587	17,108	926	963	0.96
Average		5.03	16.10	22,950	17,177	856	813	1.08
Pike's Peak	6, 16	7.29	13.36	23,720	13,188	956	1,009	0.95
Pike's Peak	6, 17	7,10	14.40	25,412	14,148	999	904	1.11
Pike's Peak	6, 18	6.18	14.90	25,272	14,005	860	840	1.02
Pike's Peak	6, 19	6.37	14.71	27,280	14,731	932	912	1.02
Pike's Peak	6, 20	5.69	15.36	27,684	14,685	830	815	1.02
Pike's Peak	6, 21	6.29	15.20	25,084	13,643	843	763	1.10
Pike's Peak	6, 22	5.77	14.52	23,928	13,108	751	864	0.87
Pike's Peak	6, 23	6.69	15.10	25,604	14,028	933	787	1.19
Average		6.42	14.69	25,498	13,942	888	862	1.05
Ross								
Colo. Springs	6, 1	4.76	16.39	26,573	19,606	925	880	1.05
Colo. Springs	6, 8	4.87	16.34	18,106	13,683	661	618	1.07
Colo. Springs	6, 9	5.01	16.37	19,330	14,538	723	647	1.12
Colo. Springs	6, 11	5.42	16.17	24,820	18,476	994	848	1.17
Average		5.02	16.32	22,207	16,576	826	773	1.10
Pike's Peak	6, 17	6.16	14.14	26,884	14,972	916	1,042	0.88
Pike's Peak	6, 18	5.44	15.51	26,796	14,847	802	805	1.00.
Pike's Peak	6, 19	5.44	15.91	31,816	17,204	929	846	1.10
Pike's Peak	6, 20	4.97	16.57	33,328	17,992	887	757	1.17
Pike's Peak	6, 21	5.12	15.83	31,112	16,898	858	882	0.97
Pike's Peak	6, 22	4.59	16.43	29,024	16,348	744	732	1.02
Pike's Peak	6, 24	5.19	15.64	29,818	16,300	839	867	0.97
Average		5.47	15.92	29,825	16,366	853	849	1.02

TABLE 2—*Concluded*

PLACE	DATE	CO_2	O_2	VOLUME OF AIR PER MINUTE AT PREVAILING BAROMETER	VOLUME OF AIR AT 0° AND 760 MM.	CO_2 EXHALED PER MINUTE	O_2 ABSORBED PER MINUTE	R.Q.
Schneider								
	1916	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
Colo. Springs	5, 25	4.11	16.77	19,013	14,041	571	587	0.97
Colo. Springs	6, 2	3.93	16.74	23,550	17,557	683	750	0.91
Colo. Springs	6, 9	4.19	16.44	24,740	18,550	770	850	0.91
Average		4.06	16.65	22,434	16,716	675	729	0.93
Pike's Peak	6, 17	5.27	15.60	21,048	11,760	615	634	0.97
Pike's Peak	6, 18	4.81	15.95	27,784	15,151	723	762	0.95
Pike's Peak	6, 19	4.67	15.88	25,704	13,966	647	721	0.90
Pike's Peak	6, 20	4.80	15.27	24,864	13,559	645	800	0.81
Pike's Peak	6, 21	4.86	15.95	26,384	14,308	690	718	0.96
Pike's Peak	6, 22	4.82	16.14	27,904	15,133	723	725	1.00
Pike's Peak	6, 23	4.57	16.09	25,600	14,080	638	694	0.92
Pike's Peak	6, 24	5.17	15.66	27,015	14,796	759	784	0.96
Average		4.87	15.82	25,788	14,094	680	730	0.93

output at the low and high altitudes respectively ranged as follows: Cheley, 221–263 cc. and 213–274 cc.; Ross, 222–278 cc. and 219–308 cc.; Schneider, 170–210 cc. and 157–225 cc. The consumption of oxygen ranged at the two altitudes as follows: Cheley, 231–310 cc. and 252–310 cc.; Ross, 247–308 cc. and 229–304 cc.; and Schneider, 183–220 cc. and 178–250 cc.

The gaseous exchange during a standard exercise on a bicycle. A summary of our data obtained during the very moderate exercise on the bicycle is given in table 2. Here again there was not a clear increase in the gaseous exchange at the high altitude. Cheley and Ross gave an average rise in the carbon dioxide output of 3.7 and 3.3 per cent respectively; while their increase in the consumption of oxygen, when we omit the first determination at the high altitude for each, averaged 3.4 per cent for Cheley and 5.4 per cent for Ross. Schneider showed no change with this amount of exercise. The increase observed in the two cases was in the direction reported by others; but was considerably less than the increase reported by Zuntz, Durig and other investigators of this subject. The ranges of the exercise exchanges in carbon dioxide and oxygen clearly fail to indicate a permanent rise in the level of the

metabolism during exercise at the high altitude. The carbon dioxide output at the low and high altitudes respectively ranged as follows: Cheley, 826–926 cc. and 751–999 cc.; Ross, 661–994 cc. and 744–929 cc.; Schneider, 571–770 cc. and 615–759 cc. The consumption of oxygen, with the exception of that recorded on the first day of residence on Pike's Peak for Cheley and Ross, ranged as follows: Cheley, 700–963 cc. and 763–912 cc.; Ross, 618–880 cc. and 732–882 cc.; Schneider, 587–850 cc. and 634–800 cc.

Our first determinations of the respiratory exchanges on Cheley were made at 9 o'clock in the evening, 10 hours after we arrived on the summit of the mountain. At that time, when at rest in the sitting posture, his metabolism was somewhat under his average for Colorado Springs; while during the standard exercise it was considerably above his normal. In exercise the output of carbon dioxide was 100 cc., or 11.7 per cent, above normal; while the consumption of oxygen was 196 cc., or 24.1 per cent, above normal and 97 cc. more than at any other time during the sojourn on Pike's Peak. The next morning at 11 o'clock, 24 hours after our arrival, the metabolism in exercise was still somewhat above normal; but the consumption of oxygen had fallen off by as much as 105 cc. It was back to normal in all determinations made on succeeding days. Ross likewise showed a high rate of metabolism during exercise at the time of the first determinations, which was made 23 hours after his arrival on the summit. His consumption of oxygen was then 160 cc. more than at any other time. This high exercise level occurred when the gaseous exchange for the sitting position was no greater than the average at the low altitude. At the time of the second experiment made on Ross on Pike's Peak, 48 hours after his arrival, the metabolism was within the limits of his high altitude normal. The first determinations on Schneider in exercise were within the range of his low altitude normal. It is well to repeat in this connection a statement made above; namely, that Schneider's gaseous exchange for the sitting posture was somewhat above normal during the first two days spent on the mountain. It appears, therefore, that there is a tendency for the metabolism to be increased during the first day or two of residence at such a high altitude as 14,110 feet. This tendency may appear in the gaseous exchange during rest in some men and only during exercise in others. With the development of acclimatization, gaseous metabolism returns to normal or at best remains only slightly above normal.

The gaseous metabolism during walking at 4 miles an hour. These experiments were conducted a year earlier than those described above.

TABLE 3

Respiratory exchange, walking at the rate of 4 miles an hour

PLACE	DATE	CO_2	O_2	VOLUME OF AIR PER MINUTE AT PREVAILING BAROMETER	VOLUME OF AIR AT 0° AND 760 MM.	CO_2 EXHALED PER MINUTE	O_2 ABSORBED PER MINUTE	R.Q.
Havens								
	1914	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
Colo. Springs	6, 5	5.39	15.26	31,075	23,166	1,239	1,332	0.930
Colo. Springs	6, 12	5.03	15.83	29,585	22,349	1,115	1,147	0.972
Colo. Springs	8, 10	4.46	15.93	32,030	24,195	1,069	1,246	0.858
Colo. Springs	8, 11	4.55	15.68	32,580	24,650	1,112	1,341	0.829
Colo. Springs	8, 24	4.76	15.17	28,960	21,842	1,031	1,317	0.783
Average		4.84	15.57	30,846	23,240	1,113	1,277	0.874
Pike's Peak	6, 17	5.94	14.10	37,060	20,781	1,226	1,470	0.834
Pike's Peak	6, 18	6.06	13.84	33,210	18,628	1,121	1,418	0.790
Pike's Peak	6, 19	6.25	13.77	29,585	16,543	1,027	1,226	0.838
Pike's Peak	6, 20	5.89	13.72	34,585	19,357	1,132	1,465	0.772
Pike's Peak	6, 24	5.80	14.38	34,130	19,103	1,100	1,291	0.852
Pike's Peak	6, 26	5.47	14.29	37,430	20,740	1,126	1,444	0.780
Pike's Peak	6, 29	5.44	14.57	32,910	18,459	1,004	1,220	0.823
Average		5.83	14.09	34,130	19,090	1,105	1,362	0.811
Schneider								
Colo. Springs	6, 11	5.32	15.76	34,235	25,817	1,365	1,327	1.027
Colo. Springs	6, 12	5.18	15.53	31,810	24,029	1,235	1,314	0.939
Colo. Springs	7, 20	4.83	15.67	30,010	22,595	1,082	1,216	0.889
Colo. Springs	8, 10	4.60	15.51	28,370	21,431	977	1,209	0.808
Colo. Springs	8, 11	4.46	15.67	28,890	21,858	966	1,198	0.806
Colo. Springs	11, 10	4.94	15.69	29,520	21,970	1,077	1,171	0.920
Average		4.89	15.64	30,472	22,950	1,117	1,239	0.898
Pike's Peak	6, 16	5.24	14.51	39,785	22,268	1,158	1,501	0.771
Pike's Peak	6, 17	5.62	14.11	40,285	22,596	1,261	1,627	0.775
Pike's Peak	6, 18	5.88	14.64	38,610	21,656	1,265	1,388	0.911
Pike's Peak	6, 19	5.22	15.09	35,635	19,902	1,031	1,196	0.862
Pike's Peak	6, 20	5.78	14.71	39,055	20,859	1,197	1,322	0.905
Pike's Peak	6, 24	5.79	14.20	33,905	18,977	1,091	1,326	0.823
Pike's Peak	6, 26	5.76	14.64	33,530	18,686	1,069	1,203	0.889
Pike's Peak	6, 29	5.39	14.78	35,860	20,114	1,076	1,279	0.841
Average		5.59	14.59	37,083	20,633	1,145	1,355	0.847

TABLE 3—*Concluded*

PLACE	DATE	CO_2	O_2	VOLUME OF AIR PER MINUTE AT PREVAILING BAROMETER	VOLUME OF AIR AT 0° AND 760 MM.	CO_2 EXHALED PER MINUTE	O_2 ABSORBED PER MINUTE	R.Q.
Sisco								
	1914	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
Colo. Springs	6, 5	5.05	15.67	36,150	26,950	1,350	1,442	0.936
Colo. Springs	6, 11	5.02	15.12	25,685	19,402	966	1,170	0.826
Colo. Springs	6, 12	5.04	15.93	35,185	26,579	1,329	1,326	1.002
Colo. Springs	8, 24	4.48	15.86	31,625	23,890	1,070	1,249	0.857
Colo. Springs	8, 24	4.69	15.09	29,560	22,294	1,037	1,371	0.756
Average		4.86	15.53	31,641	23,823	1,150	1,312	0.875
Pike's Peak	6, 16	6.25	14.22	41,060	22,981	1,427	1,572	0.908
Pike's Peak	6, 17	5.76	14.37	41,120	23,064	1,319	1,564	0.843
Pike's Peak	6, 18	5.27	14.57	40,730	22,845	1,195	1,521	0.786
Pike's Peak	6, 19	5.72	14.23	38,260	21,368	1,214	1,489	0.815
Pike's Peak	6, 20	5.66	14.14	37,270	20,860	1,172	1,481	0.791
Pike's Peak	6, 22	5.17	14.50	36,410	20,288	1,041	1,373	0.758
Pike's Peak	6, 24	5.67	14.94	38,880	21,761	1,225	1,323	0.926
Pike's Peak	6, 26	6.16	14.19	36,560	20,371	1,247	1,406	0.887
Pike's Peak	6, 28	5.29	14.85	37,155	20,796	1,092	1,310	0.834
Average		5.66	14.45	38,605	21,593	1,215	1,449	0.839

The exercise consisted of indoor walking on a smooth floor at the rate of 4 miles an hour. The courses at the two altitudes were carefully measured off, but differed in respect to the number of turns that had to be made by the walker. In Colorado Springs the course was straight; while on Pike's Peak a tortuous course was laid out in two rooms, one of which was heated and the other cold. These conditions make the walking experiments less satisfactory for comparison than the others we have reported. The data for these experiments are summarized in table 3. One of the three subjects, Schneider, had also served in the other experiments. In him this form of exercise which demanded more energy than the work on the bicycle, resulted in an increased metabolism during the first 48 hours spent at the high altitude. The first determination was made 6 hours after his arrival on the summit. At that time the carbon dioxide output was only slightly above the low altitude average, but the oxygen consumption was markedly above any of his low altitude records. The next morning, approximately 24 hours after

his arrival, the metabolism was even greater; at that time the oxygen consumption was 31.3 per cent above his low altitude average. The next day, after a sojourn of 48 hours, the metabolism was less, but not back to normal. For the first 48 hours of residence at the high altitude the output of carbon dioxide averaged 9.9 per cent and the oxygen consumption 21 per cent above the low altitude averages. During the following 11 days of further residence on the Peak the gaseous metabolism in walking was approximately the same as at the low altitude.

While at the high altitude Havens showed an unevenness in the consumption of oxygen; it was abnormally high during the first two days and then later was fully as low as in Colorado Springs, but on two occasions it again came up to that observed during the first days. Haven's oxygen consumption while on Pike's Peak averaged 6.6 per cent above that of Colorado Springs. On the other hand, the carbon dioxide output was at no time above that of the low altitude; in fact it averaged practically the same at the two altitudes.

The first determination on Sisco was made 7 hours after his arrival on the summit. The exchanges of both carbon dioxide and oxygen were then above the low altitude normals, 24 and 20 per cent respectively. The exchange was still high at the end of 24 hours. The output of carbon dioxide was back to normal in 48 hours; but the consumption of oxygen did not fall as much, and clearly remained above normal during the first five days of the stay on Pike's Peak. In fact, his consumption of oxygen during a residence of 12 days was never as low as in some tests in Colorado Springs. While Sisco was on the Peak the carbon dioxide output averaged 5.7 per cent and the consumption of oxygen 10.4 per cent above those for Colorado Springs. It appears, therefore, that in this case the high altitude caused a clear and definite increase in the gaseous metabolism during exercise.

Our two series of physical exercise experiments show that, during the first part of a sojourn at a high altitude, physical exertion may be more costly to the body than at a low altitude or than it will be later on at the high altitude. As acclimatization occurs, the gaseous metabolism usually returns to, or almost to, the low altitude level. In some individuals this return is made more slowly than in others.

The respiratory quotient throughout our three series of metabolism experiments was not definitely altered by residence at the high altitude. This also supports the conclusion that there was not a profound change in the gaseous exchange at the high altitude. In nearly all of the work experiments with Cheley and Ross, the respiratory quotient is 1 or

above. This abnormally high value was the result of collecting the exhaled air too soon after the exercise began.

Other respiratory conditions of interest are found in the data given in tables 1 and 2 which show that individuals do not all respond to the altitude in exactly the same way. The volume of air breathed per minute is, as a rule, somewhat increased by residence at a high altitude. In Cheley, however, when sitting at rest the average per minute volume was the same on Pike's Peak as in Colorado Springs. The first observation made on him on Pike's Peak showed an unusually small minute-volume; but at the time of the second determination, 24 hours after his arrival, the volume was back to his low altitude normal and was maintained at that point. In exercise his minute-volume was greatest at the high altitude. During the first work experiment this was only slightly above his average for Colorado Springs and not yet up to the high altitude normal, which was reached by the end of the first 24 hours of residence on the mountain.

Ross, both when sitting quietly and exercising, had a larger minute-volume on Pike's Peak than in Colorado Springs. His average increase for the sitting posture was 22.9 per cent and for exercising, 34.3 per cent. Cheley in exercise showed an altitude increase of only 11.1 per cent. Schneider's per-minute volume increase stood between these, with an average of 10.1 per cent for the sitting posture and 14.9 per cent in exercise. Ross did not immediately reach the maximum volume of breathing, but required three days, instead of one as did Cheley, to reach his normal for Pike's Peak. Schneider reached his high altitude minute-volume during rest within 24 hours, but the normal for exercise was not established for another 24 hours.

In passing it should be observed that the percentages of carbon dioxide and oxygen in the exhaled air changed with acclimatization. The carbon dioxide content showed a slight decrease and the oxygen an increase. Furthermore Cheley, who had the least minute-volume change in his breathing, persistently showed a lower content of oxygen in the exhaled air than did Ross and Schneider.

ALVEOLAR AIR. In earlier studies (15), (16), (17) we have compared the effects upon the hemoglobin and erythrocyte content of the blood and upon the pulse rate of repeated journeys to the summit of Pike's Peak; and also considered the influence upon these of physical fitness, of climbing the mountain afoot, and of mountain sickness. There are among our unpublished data a considerable number of series of alveolar air determinations that make it possible to raise the question as to how

the above mentioned conditions may influence the ventilation of the lungs, insofar as these may be determined from analyses of the alveolar air.

All of the observations here considered were made under resting conditions, and the sampling of the alveolar air was by the direct method of Haldane and Priestley (11). Much of the time two samples were analyzed and the results averaged. All analyses were made with the Haldane gas analysis apparatus. The observations were made on seven subjects, of these there are four series on Schneider, three on Havens, two on Sisco and one on each of four other men.

As indicated by our data on the total respiratory exchange, and as has long been known, an ascent to a high altitude causes an increase in the volume of air breathed and at the same time a lowering of the partial pressure of carbon dioxide in the alveolar air. It has been found, in an ascent to a high altitude, that the alveolar carbon dioxide pressure does not immediately fall to its new level; but that it may steadily decrease for a period of as much as 19 days (4). Different individuals react at different rates and speeds. Our own data also bring out the fact that the same individual may react differently in several ascents to the same altitude.

The data obtained from a study of Schneider in four expeditions give an opportunity to consider factors that may explain the cause of mountain sickness. That mountain sickness is caused by a lack of oxygen can hardly be questioned, but that it is the sole cause is not the accepted theory. Barcroft (1) believes that mountain sickness is induced by the action of want of oxygen on the brain, and that the vomiting center is stimulated by the lack of acid in the blood. Haldane, Kellas and Kennaway (12) and Sundstroem (18) hold that at least a secondary cause is an abnormally high proportion of alkali in the blood. Y. Henderson and Haggard (10) suggested that the hemato-respiratory events of acclimatization which lead to an alkalosis of the blood are as follows: *1*, a lowering of the oxygen tension of the inspired air; *2*, stimulation of the respiratory center by oxygen want; *3*, excessive respiration, with a blowing off of carbon dioxide from the blood; and *4*, a decrease in the ratio of H_2CO_3 to $NaHCO_3$, which of course gives an alkalosis. A still later event of acclimatization calls for the excretion of the excess of alkali from the blood. If alkalosis of the blood is an important factor in the production of mountain sickness, the alveolar air analysis ought to give evidence as to its degree of importance as a cause.

The data for Schneider, which are tabulated in table 4, will also be considered from the standpoint of physical fitness. The first series of observations were made on Schneider during the Anglo-American Expedition of 1911 (4). Prior to the expedition he had not taken physical exercise for a period of 8 months and had only recently come up from sea-level to Colorado Springs, altitude 6000 feet; and consequently, was not wholly acclimated to that altitude when the ascent of Pike's Peak was made. On the whole he was in rather poor condition. During the winter and spring preceding the expedition of 1913, in which the second series of observations was made, he had occasionally climbed in the mountains and was in fair condition. Preceding the 1914 expedition he had played tennis regularly several times a week for 3 months and had also frequently hiked in the mountains. In 1916 he had played some tennis and done some mountain climbing but not as regularly as in 1914. He was in better condition during the last two than in the two earlier expeditions. During the first sojourn on Pike's Peak, 1911, Schneider suffered from mountain sickness for a number of days, during which time he had a severe headache and nausea on the first day. He had, in 1913, an attack of mountain sickness, but was much improved after the first night; although all symptoms did not disappear for 2 days. In 1914 he felt quite well throughout the stay on the summit, and in 1916 was well except for nausea and a headache that were experienced the first evening and night. Mountain sickness was most severe during those expeditions in which this subject was in the poorest physical condition, and the one time that he escaped was preceded by a season of regular exercise and good health.

An examination of Schneider's alveolar air records shows that the changes in these follow a fair parallelism with the experience of mountain sickness. In 1911, the 9 days' residence at 6000 feet had not been sufficient to bring the alveolar carbon dioxide and oxygen tensions to the normal for that altitude. The mean alveolar carbon dioxide pressure for acclimatized men has been reported by the Anglo-American Pike's Peak Expedition (4) and by Miss Fitzgerald (8) as 33.85 mm. in Colorado Springs (6000 feet), and 27 mm. on Pike's Peak (14,110 feet). When Schneider ascended the Peak in 1911 the respiratory adaptive response was unusually slow. His alveolar carbon dioxide tension fell in 24 hours from the unusually high average of 37.4 mm. to only 36.1 mm., and to 34.6 mm. in 48 hours, and at no time in a stay of 5 weeks was it as low as in later expeditions. If a blowing off of carbon dioxide is necessary to produce the alkalosis that is predicated in the early stay

TABLE 4

Composition of alveolar air on Pike's Peak in milligram Hg

TIME	SCHNEIDER								HAVENS						SISCO				CHELEY		ROSS	
	Carbon dioxide				Oxygen				Carbon dioxide			Oxygen			CO_2		O_2		CO_2	O_2	CO_2	O_2
	1911	1913	1914	1916	1911	1913	1914	1916	1912	1913	1914	1912	1913	1914	1913	1914	1913	1914	1916	1916	1916	1916
Colo. Springs, Av.	37.4	33.9	34.0	33.8	75.7	82.9	81.2	81.1	34.3	35.5	35.8	78.7	77.5	75.3	32.9	33.7	82.1	78.6	34.1	78.8	32.6	75.6
3 to 7 hours	34.2		28.0	31.8	43.9		52.4	46.6	31.9	34.9	34.6	46.5	45.4	43.3	32.8	32.1	44.5	46.2	35.3	42.2	29.8	48.2
1 day	36.1	33.2	28.4	28.0	40.2	44.4	51.6	50.8	29.9	33.1	29.7	48.3	48.9	49.7	31.3	31.7	44.5	45.5	32.1	46.4	29.6	52.0
2 days	34.6	28.2	30.2	27.8	46.5	52.6	46.0	52.0	28.1	31.7	29.8	49.4	49.0	50.9	30.6	28.1	48.7	47.5	30.2	51.1	27.9	53.5
3 days	31.9	29.4	30.2	27.4	47.7	52.4	49.0	50.1			29.8			48.6	29.5	29.2	49.3	51.0	29.9	47.6	27.3	52.4
4 days	32.8	26.4	30.4	28.6	47.3	54.9	45.4	51.4		29.7	30.7		51.3	47.2	30.0	29.8	49.2	47.3	26.9	54.8	23.9	57.1
5 days	32.0	28.7	29.0	27.0	46.1	51.0	48.5	50.6		29.3	27.2		52.3	55 5		27.9		49.7	26.8	52.6	25.2	59.3
6 days	31.5		27.3	26.8	48.2		51.0	55.3			28.6			52.5		28.3		52.7	27.2	50.2	23.4	56.8
7 days	30.9		25.7		54.9		55.2				28.1			53.6		27.8		53.1	28.2	51.5	26.2	55.7
8 days	30.8		29.2		52.2		52.0									27.2		53.7				
9 days	30.6		28.0		53.8		49.6				26.9			53.0		28.2		52.0				
10 days	30.8		28.6		51.2		52.1				27.6			54.2		28.3		51.6				
11 days			25.9				55.7				27.0			53.4		28.4		48.2				
12 days	31.0		27.2		54.6		53.1				25.4			54.6								

at a high altitude, then it appears that Schneider had not as yet developed an alkalosis at a time that he was suffering from mountain sickness. In 1913, the alveolar carbon dioxide showed practically no change in 24 hours, during a part of which time the subject felt very uncomfortable; but by the end of 48 hours when he was feeling better, the alveolar carbon dioxide had fallen 5.7 mm. In 1914, when he was not mountain sick, 5 hours after arriving on the summit the carbon dioxide tension had already been lowered from 34 mm., his average in Colorado Springs, to 28 mm. Such a sudden fall in the carbon dioxide makes an alkalosis probable. In 1916, the alveolar carbon dioxide content again fell more slowly, from 33.8 to 31.8 mm. in 7 hours; and during and immediately following this period there occurred a brief attack of several hours of mountain sickness, which had entirely disappeared by the end of the first 24 hours, when the alveolar carbon dioxide had further fallen to 28 mm.

The differences in Schneider's health, observed in the four expeditions, appear to have been more closely associated with an oxygen deficiency than with an alkalosis. An inspection of table 4 shows that the alveolar oxygen tension was lowest during the periods when mountain sickness symptoms were most in evidence. In 1911, the oxygen tension was only 40.2 mm. at the end of the first 24 hours and then gradually and slowly increased to 54.4 mm. during the next six days. In 1913, the alveolar oxygen was 44.4 mm. at the end of the first 24 hours, and 52.6 mm. in 48 hours; in 1914, it was 52.4 mm. within 5 hours, 51.6 mm. at 24 hours, and then somewhat lower the following days. In 1916, the oxygen tension was 46.6 mm. at the end of the first 7 hours and 50.8 mm. at 24 hours.

The above data fail to associate the symptoms of mountain sickness, as experienced by Schneider, with a marked or rapid lowering of the alveolar carbon dioxide which, according to the theory of the production of an alkalosis, should be necessary if an important cause of mountain sickness is an alkalosis of the blood. They do, however, indicate that oxygen want is an important causative factor.

In the three series of observations on Havens we have an opportunity of learning whether regular athletic training puts a man in condition to make the respiratory response more quickly, and also whether climbing the mountain afoot modifies the adaptive respiratory process. Prior to the 1912 series Havens had worked out-of-doors all summer at manual labor; in 1913 he had trained daily for a period of 3 months for the 2-mile run of intercollegiate track meets; and in 1914 he had again trained for the 2-mile run, but discontinued regular running about 3½ weeks before

the trip to Pike's Peak. The 1912 ascent was made afoot and the other two ascents by railway train. Havens did not experience mountain sickness in any of these expeditions. And yet, when the data for his alveolar air changes in carbon dioxide and oxygen are compared with those of Schneider, we find there is no decided difference present in the rate and degree of change. He made the changes in 1913 and 1914 more slowly than did Schneider in his sojourns of 1914 and 1916, when he suffered least from mountain sickness. Havens appears to have been somewhat more tolerant of low oxygen than Schneider.

Within 30 minutes after Havens reached the summit, after walking up Pike's Peak, the first determination of alveolar carbon dioxide was made; at this time there was a fall from 34.3 mm., his Colorado Springs average, to 31.4 mm. This small lowering of the carbon dioxide tension indicates that there had not been a great formation of lactic acid as a result of the muscular exertion involved in the climb. The second alveolar air determination, made 3 hours later, showed 31.9 mm. of carbon dioxide and 46.5 mm. of oxygen. By the following morning the carbon dioxide was further lowered to 29.9 mm., and the oxygen raised to 48.3 mm.; which values were quite like those obtained at the end of a similar period of sojourn during the other expeditions. Two other subjects, who walked up the Peak in 1914 and remained 4 days, showed the same reaction as did Havens after the climb afoot. So it appears that the exertion of walking a distance of 9.8 miles with a rise of 7485 feet did not materially modify the rate or the degree of change in the alveolar carbon dioxide and oxygen at the high altitude.

There is nothing whatever in the observations on Havens to indicate that the excellent physical condition resulting from athletic training rendered the organism more capable or more effective in making the altitude respiratory adaptive changes. In this the observations on him differ from those on Schneider. Neither does the fatigue resulting from climbing the mountain afoot clearly modify the rate or degree of the respiratory change. In this the altitude respiratory adaptive changes differ from the altitude changes in the hemoglobin and red corpuscles of the blood. Havens and Schneider (15) have shown that the fatigue of walking up a mountain delays the altitude increase in hemoglobin and red corpuscles, and that the altitude increase in these blood elements is most rapid in men who are in excellent physical condition.

The data for Sisco, table 4, also show that some individuals are more tolerant of low oxygen than others. In his two sojourns, for which the

data are complete, he was troubled during the first, 1913, with a headache which may have been caused either by a cold or by the altitude; while during the second expedition, 1914, he was very well. In 1913, the alveolar carbon dioxide changed slowly so that a pronounced alkalosis could not have occurred. His average carbon dioxide tension was 32.97 mm. in Colorado Springs, 32.8 mm. 4 hours after his arrival on the summit of the Peak, and 31.3 at the end of the first 24 hours. In 1914, when he felt no altitude effects, the Colorado Springs alveolar carbon dioxide averaged 33.7 mm.; and 2½ hours after reaching the summit it was about the same, 33.2 mm. After a stay of 24 hours the two sojourns show almost identical determinations; namely, 31.3 and 31.7 mm. Sisco's alveolar oxygen tension during the first hours of residence on Pike's Peak was higher in 1913 than in 1914; being 44.5 in 1913 and 41.7 mm. in 1914. Schneider was mountain sick with an alveolar oxygen tension of 40.2 and 44.4 mm.; while Sisco was entirely comfortable, in 1914, with an oxygen tension of only 41.7 mm. Just why Sisco should have been ill in 1913 is not evident from the records of either the carbon dioxide or oxygen. It is suggestive to find that in 1913 his hemoglobin in 24 hours rose only from 111 to 116 and in 1914, from 113 to 120 per cent (15).

Cheley and Ross, whose records also appear in table 4, were members of the 1916 expedition. Cheley had a headache during the first afternoon and night, but felt no other symptoms of mountain sickness. His alveolar carbon dioxide which was 35.3 mm. during the first hours on the Peak was slightly higher than in Colorado Springs, where it averaged only 34.1 mm. There is then no evidence of an alkalosis. His alveolar oxygen pressure was only 42.2 mm. during the first eight hours, 46.4 mm. at the end of 24 hours, and 51.1 mm. at 48 hours. Oxygen want seems to account for his illness.

Ross had a headache for several days but no nausea or other disturbances. An explanation of his case is not as clearly found in his respiratory record as in others. His minute volume increase in breathing, both in rest and during exercise, was greater, while on Pike's Peak, than in Cheley and Schneider. In Ross, after reaching the summit, the resting alveolar carbon dioxide tension fell in 5 hours from an average of 32.6 mm. to 29.8 mm., and to 29.6 mm. by the following morning. This fall in carbon dioxide indicates a slight degree of alkalosis of the blood, but that it caused the symptoms of mountain sickness does not seem a likely explanation in view of observations made under low barometric pressure in a low pressure chamber. Lutz and Schneider

(14) in such experiments found that a stay of several hours at a barometric pressure of 380 mm., which caused the alveolar carbon dioxide to fall from 38.9 to 30.3 mm., did not cause symptoms of mountain sickness. Furthermore, our observations on Schneider show that, in 1914, when he was not affected by the altitude sickness, his alveolar carbon dioxide fell from 34 to 28 mm. Ross had a fairly high alveolar oxygen tension, 48.2 mm., during the first half-day spent on the Peak, and 52 mm. by the end of the first 24 hours. After recovery from the headache his alveolar oxygen averaged higher than any other subject; namely, between 55.7 and 59.3 mm. These data make it appear that Ross was less tolerant of low oxygen than the other men we have studied.

Our alveolar air data lead to the conclusion that some men are more tolerant of low oxygen than others and that mountain sickness is more closely associated with oxygen want than with an alkalosis of the blood.

It was pointed out by the Anglo-American Expedition (4) that if the same alveolar carbon dioxide pressure were maintained on Pike's Peak as at sea level the pressure of oxygen would be only 35.8 mm., but that parallel with the fall in alveolar carbon dioxide there is an average rise of 16.2 mm. in the alveolar oxygen tension, so that in acclimatization the alveolar oxygen pressure is 52 mm. instead of 35.8 mm. Our subjects gave typical responses in that they, as a rule, gradually increased the alveolar oxygen pressure. The time required to reach a pressure of 52 mm. varied with individuals and with the same individual at different times. Cheley reached the 52 mm. alveolar oxygen pressure on the 4th day of residence and went no higher during another 4 days. Havens reached it in 4 days in 1913 and in 5 in 1914, in acclimatization in 1914 his alveolar oxygen averaged about 54 mm. For Sisco 52 mm. was about maximal and was reached in 6 days. Schneider showed more variation than others, reaching 52 mm. almost at once in 1914, in 7 days in 1911, and in 2 days in 1913 and 1916. His alveolar oxygen tension varied from time to time between 49 and 55 mm. Ross went to 57.1 mm., about his acclimatization average, in 4 days.

SUMMARY

1. The respiratory exchange was determined throughout the period of acclimatization during a sojourn on Pike's Peak, altitude 14,110 feet, in men sitting at rest, during a moderate amount of work on a bicycle, and during walking at the rate of 4 miles an hour. Two subjects in rest experienced no change, or at the most only a slight increase, in their gaseous metabolism; while in a third subject the high altitude

resting metabolism was above that of the low altitude for 48 hours, during which time the increase averaged for carbon dioxide 14.2 per cent and oxygen 17.6 per cent. After this he returned to his low altitude metabolism level.

Five men were under observation during exercise. Physical exertion caused a higher rate of metabolism than was normal for the low altitude during the first days of residence at the high altitude. There were individual differences in the amount of increase and in the time that the heightened metabolism persisted. All except one subject returned to the rate of metabolism normal for the low altitude within 1 to 3 days. The exceptional case maintained his normal low altitude level while at rest, but during exercise at the high altitude in 13 days never quite returned to his low altitude normal.

2. The changes in the composition of the alveolar air were followed in seven subjects, in one of these in four, in a second in three, in a third in two and in the others in one expedition each. There occurred individual differences, and differences in the same individual in several ascents, in the rate and extent of change. The changes were accomplished within from a few hours to 7 days. In one subject, studied in four expeditions, the rate of change varied with physical condition and was most rapid following a period of regular indulgence in exercise. Climbing the mountain afoot did not affect the respiratory acclimatization process.

3. Mountain sickness was found to be most severe in the physically unfit and to be associated with a low alveolar oxygen tension. Some individuals were more tolerant of a low alveolar oxygen than others. The changes in the alveolar carbon dioxide tension did not indicate an alkalosis of the blood during the period of mountain sickness. Because of this fact, it has been concluded that it is doubtful whether alkalosis is an important causative factor in mountain sickness.

The writer desires to express his appreciation of the hearty coöperation and assistance given him by L. C. Havens, G. E. Cheley, S. B. Ross, R. M. Atwater, H B. Gregg and D. L. Sisco.

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THE EFFECT OF EXERCISE ON VITAMIN REQUIREMENTS

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Received for publication February 26, 1923

There is a more or less prevalent impression that the ill effects of lack of vitamins are more severe or appear earlier in both animals and human subjects under the strain of muscular exercise than in comparable subjects at rest, but no experimental evidence in support of this impression has been reported. The belief is based mainly upon somewhat questionable *a priori* grounds and uncontrolled observations of human experiences in deficiency diseases.

The report of the British Medical Research Committee (1), summing up the case says: "There are some grounds for believing that the accessory food factors are concerned in the process of tissue formation and repair, and we should therefore expect larger amounts to be necessary after exercise than after rest. Whilst there is as yet practically no experimental evidence upon this point, there are indications based upon human experience that the demand for the three accessory factors is greater when work is being accomplished." The experiences to which they refer in detail relate only to scurvy.

McCarrison (2) reports that polyneuritis developed more quickly and was more quickly fatal in pigeons congregated in large cages than in segregated pigeons, and he attributes the difference mainly to the greater physical fatigue of the birds which were more active and more excited by one another. He says: "The demand for vitamins appears to be greater the greater the physical work; a conclusion which is justified as much by clinical experience as by experimental results. Thus the onset of human beriberi is often rendered acute, or that of hunger oedema sudden, by excessive physical exertion. Further, the incidence of a deficiency disease, such as beriberi or pellagra, has been shown to be highest among those whose work is hardest."

In order to put this question to an experimental test we have compared the condition and the weight changes of exercised and unexercised rats on rations which were deficient in vitamin A or vitamin B, other conditions being kept as nearly uniform as possible.

GENERAL PLAN AND APPARATUS. The rats were divided into pairs, one of each pair being exercised daily and the other kept in the limited space of a small cage and used as a control. The food given was weighed

daily, and fresh distilled water was supplied at all times. Although some rats scattered food occasionally, the amounts lost were small and a fairly accurate measure of the food intake was maintained. The amounts of food given to the two rats of each pair were regulated as explained below. The effects of the lack of vitamin and of the exercise were judged by the weight curves and the survival period of the rats and by the appearance of recognized deficiency symptoms. The animals were weighed individually three times a week.

The rats to be exercised were put into cylindrical wooden cheese boxes made to revolve uniformly around a horizontal axis by means of an electric motor. By the use of pulleys and a reducing speed gear, the speed of revolution of the boxes was adjusted to about 13 revolutions per minute. The box covers served as doors. To provide for ventilation, the flat portion of these covers was replaced by wire netting, the backs of the cages were perforated with several one-inch holes, and an electric fan was placed before the revolving boxes. The wire netting also provided for observation of the animals while they were exercising. The diameter of the boxes and the rate of revolution were such as to make the rats travel at the rate of about one-half mile an hour. The duration of the exercise was made short at first and gradually increased as the animals became accustomed to it, and finally amounted to about 6 or 7 hours (in two periods with rest between) on the working days, with a shorter time on Saturday and Sunday, making about 33 to 40 hours a week. The rats were not purposely exercised to the point of fatigue, although on two or three occasions an individual rat was overcome. As individual rats became enfeebled so as not to be able to endure the work, the amount of exercise was reduced for them or stopped.

The rats showed some tendency to slide as the box revolved rather than walk. If this was persisted in, narrow cleats were nailed to the box across their path. Usually, however, the animals, especially the young, would walk, and in these cases better results were obtained without the cleats. The cleats were liable to give the animals falls, because they would cling to them until carried a part of the way around. Such falls, and the fear or anxiety shown at first because of the strange conditions, introduced features which may have altered the physical condition of the animals. Life in the box also necessarily imposed conditions, such as several hours' deprivation of water and of the usual daytime sleep, which may complicate somewhat the interpretation of results; but it is believed that muscular exercise formed the main feature which would be likely to alter the vitamin requirements of the working as compared with the resting subjects.

At first it was supposed that the exercised rats would consume a larger amount of food than the controls to provide the energy for the extra muscular work, but observations on the voluntary food intake of the exercised as compared with the unexercised rats in the first days of the trial showed that such was not the rule. Accordingly, no special allowance was made generally for the energy requirement of the work imposed, but the food was adjusted for each pair approximately in proportion to the body weights. At times, however, a wide discrepancy between the weights of the two animals of a pair accompanied by evidence of an unsatisfied appetite in the exercised animal was met by an addition to the amount of food given to the exercised rat. In case one animal of a pair refused food because of an evident pathological condition, the amount given to its mate was not reduced to correspond. After the death of one of a pair the mate was allowed to eat all he would take.

During the course of the experiment, some of the animals, especially the adults, developed colds, pneumonia, or a temporary eye infection, and some slight injuries were received. All were subject alike to such exigencies, except that congregation in the exercise chamber unavoidably increased the chances for contagion. There was no evidence of such contagion unless perhaps in the case of the eye infection mentioned This eye infection was developed in the rats on vitamin B deficiency and was distinct from that developed by the deficiency in vitamin A.

EFFECTS OF EXERCISE ON ADULT AND HALF-GROWN RATS ON A COMPLETE RATION. In order to determine whether exercise such as was used in the vitamin tests would, of itself, affect the weight curves of animals on complete rations, eight pairs of adult rats and three pairs of half-grown rats received, for a longer or shorter time, complete rations similar to those of the vitamin B tests but carrying dried yeast to supply the vitamin. One rat in each pair was put to work in the revolving box, while the other rat was confined in a small cage.

The rations fed to these rats and to those in the vitamin B tests were all on a 15 per cent protein basis and consisted of casein or beef proteins, the Osborne and Mendel salt mixture, butterfat, and either patent white flour, polished rice or cornstarch with a small amount of cane sugar in some cases and with 3 percent of agar-agar except when the rice was used. Two per cent of yeast replaced a like amount of the carbohydrate component in making the complete rations. The casein used in most of the rations was extracted thoroughly with alcohol or acidified water to remove any adherent vitamin B. In making up the rations the agar-agar was first dissolved in boiling water and then added to the other dry ingredients, which had been thoroughly mixed. More boiling water was added

to the mixture if needed to bring it to the consistency required for complete dextrinization of the starch. In order to dextrinize the starch, the mixture was cooked in a double boiler. The butterfat and yeast were then added and were intimately stirred into the hot mixture. The mass was dried at a low temperature and ground.

Four of the eleven rats used in this test were kept on the complete rations for periods of 65 to 120 days; the others, with the exception of a few that died, were transferred to the vitamin B test after shorter periods. The first section of the following table gives the weight changes of these rats, together with the duration of the several tests. Those for which the test period was less than 3 weeks have been omitted. Notice that odd numbers apply to exercised rats and even numbers to unexercised rats.

The table reveals a noticeable lack of uniformity in the results obtained with this group. This lack of uniformity is quite evident when complete growth curves are examined. Of the four pairs that were kept longest on experiment, the growth curves show a pronounced difference between the two rats of a pair only in case of rats 9 and 10. In that case the advantage was with the unexercised rat; but there was an evident, though smaller, advantage with the exercised rat in the pair, rats 13 and 14. Of the rats that were on the complete rations for shorter periods, ranging from 1 week to 5, the results show a definite advantage to the exercised rat in the case of rats 3 and 4 and to the unexercised rat in the cases of rats 5 and 6 and rats 7 and 8. In the other cases there were no marked differences. On the whole, therefore, we should say that, if the exercise in itself had an influence on growth or physical condition, it was slightly detrimental. Insofar as that is the case, it is to be borne in mind in the interpretation of the vitamin tests as detracting from any apparent effect of the exercise in increasing the vitamin requirement.

VITAMIN B TESTS. *Experiments on adult and half-grown animals.* In making the vitamin B tests such rations as have already been described were used, a more liberal allowance of butterfat and of mineral salts being made for younger animals. Six pairs of adult rats and three pairs of half-grown rats were first used. Some of these animals had previously been on experiment with the complete ration, but they had not shown any pronounced effect of the exercise such as to render them unfit for the vitamin study. The principal weight changes and the duration of the tests are shown in the second and third sections of the table.

TABLE 1

Summary of weight data

AGE	RAT		WEIGHT AT START	DURATION OF TEST	WEIGHT AT END	NET GAIN (+) OR LOSS (−) IN WEIGHT	MAXIMUM WEIGHT	MAXIMUM GAIN IN WEIGHT	LOSS FROM MAXIMUM WEIGHT
	Exercised	Unexercised							
Rats on complete ration									
			grams	*days*	*grams*	*grams*	*grams*	*grams*	*grams*
Adult and half-grown	9		163	120	115	− 48	181	18	66
		10	190		195	+ 5	220	30	25
	11		155	112*	156*	− 1	175	20	19
		12	170		179	+ 9	190	20	11
	13		202	120	240	+ 38	249	47	9
		14	223		210	− 13	247	24	37
	23		95	65	152	+ 57	157	62	5
		24	92		167	+ 75	167	75	0
	1		236	24	235	− 1	246	10	11
		2	240		248	+ 8	248	8	0
	3		165	35	188	+ 23	194	29	6
		4	145		144	− 1	149	4	5
	5		191	23	150	− 41	195	4	45
		6	222		246	+ 24	246	24	0
	7		254	21	253	− 1	258	4	5
		8	256		302	+ 46	302	46	0
Rats on rations lacking Vitamin B									
Adult	A		150	128**	104	− 46	163	13	59
		B	210	188	112	− 98	221	11	9
	3		186	70	134	− 51	186	0	52
		4	141	95†	110†	− 31	141	0	31
	7		250	168	116	−134	250	0	134
		8	302	291	123	−179	318	16	195

Adult	13		240		59		137		−103		240		0		103			
		14		210		30		129		− 81		210		0		81		
	15		215		106		143		− 72		221		6		78			
		16		262		159		110		−152		264		2		154		
	17		234		140		124		−110		234		0		110			
		18		248		119		132		−116		249		1		117		
Half-grown	19		111		57		66		− 45		112		1		46			
		20		130		182		64		− 66		130		0		66		
	21		131		300‡		170‡		+ 39		175		44		5			
		22		141		300‡		211‡		+ 70		220		79		9		
	23		152		251‡		182‡		+ 30		218		66		36			
		24		167		97		81		− 86		170		3		89		
Very young	37		46		124[d]		66[d]		+20		67		21		1			
		38		48		46		43		− 5		53		5		10		
	39		49		124[d]		104[d]		+ 55		105		56		1			
		40		44		124[d]		136[d]		+ 92		136		92		0		
	41		50		124[d]		106[d]		+ 56		114		64		8			
		42		47		73		37		− 10		61		14		24		
	43		61		117[d]		70[d]		+ 9		79		18		9			
		44		60		117[d]		147[d]		+ 87		147		87		0		
	45		55		117[d]		94[d]		+ 39		94		39		0			
		46		55		60		45		− 10		64		9		19		

* Rat 11 was exhausted in exercise on the 112th day. Eye infection had developed, and the animal appeared weak and listless. There was then a rapid loss of weight and death occurred on the 125th day at a weight of 121 grams.

** Rat A was exercised only the last 66 days. At the beginning of the 66 days he weighed 145 grams and the mate, rat B, weighed 200 grams.

† Rat 4 received yeast after 95 days. He continued to lose weight and died 75 days later at a weight of 97 grams.

‡ Of the half-grown lot, rats 21, 22, and 23 survived and the others all died in experiment.

[d] Of the very young lot, rats 37, 39, 40, 41, 43, 44 and 45 survived; only rats 38, 42 and 46 died. Rat 42 showed polyneuritis.

All of these rats died except three of the half-grown rats, rats 21, 22 and 23. These animals, being still in apparently good condition after 251 and 300 days, were then removed from experiment. The data show that in 5 of the 9 cases the exercised rat died before the unexercised; in 3 cases the exercised survived longer; and in the other case both survived for 300 days. The total decline in weight, however, was greater for the exercised rat in only 2 cases, while it was greater for the unexercised rat in 6 cases; in the pair for which the net change of each animal was a gain the growth was less for the exercised rat than for the unexercised rat. The rate of decline was more rapid in 2 cases for the exercised rat and in 3 cases for the unexercised rat, and in 4 cases the rate of change was nearly alike for the two rats of the pair. It is, therefore, evident that the weight curves of the nine pairs of adult and half-grown animals that were on rations lacking vitamin B were of quite varied types and do not give pronounced evidence of any effect of the exercise upon the vitamin B requirement of rats of this age.

With the three half-grown rats that survived the long experimental regime it was noticed that, in each case, after a maintenance period of about 100 days, there was a period of rapid growth without change of ration. Rat 23 suddenly increased her weight from 150 grams to nearly 220 grams; rat 21 increased from 110 grams to 175 grams; and rat 22 increased from 116 grams to 220 grams. The rats then appeared to be in a healthy normal condition.

Experiments on very young animals. It is generally recognized that young animals show a greater need of growth vitamins than do older animals. Special stress may, therefore, be laid on the effects obtained with animals of this age.

In our experiments five pairs of rats from two litters were fed upon the synthetic rations deficient in vitamin B from the time they were weaned, one of each pair being exercised from the first and the other not. Rats 37 and 38, 39 and 40, 41 and 42 were of one litter and rats 43 and 44, 45 and 46 were of another litter, all being started on experiment at the same age, and the exercise being begun at once. The data of the weight changes of the animals and the duration of the tests are given in the last section of the table. Three of the unexercised rats died after 46 to 73 days. The other seven rats were all still living when the experiment was terminated at the end of 117 and 124 days for the two litters, respectively.

For these very young animals the weight curves are even more varied and inconsistent than those of the adult and half-grown rats. The

pair, rat 39 and rat 40, continued to grow for months in spite of the lack of vitamin B in the ration, as did also the individual exercised animals, rats 41 and 45, and the unexercised animal, rat 44; but the pair, rats 37 and 38, and the individual exercised animal, rat 43, and the unexercised animals, rats 42 and 46, scarcely more than maintained their weight before the final decline. Rat 37, however, like rats 21, 22 and 23 before mentioned, suddenly showed a tendency to gain in weight after a period of about 100 days at practically constant weight. The rapid gain of rat 39 changed to maintenance or loss after about the same interval.

Among these young rats, the three to succumb were all unexercised, but so also were the two rats that made the most rapid and by far the greatest ultimate gain. It is evident that other factors than the amount of exercise determined the rates of growth in these young rats as well as in the older rats.

Individualities. Considering the results from all of our animals on the rations deficient in vitamin B, there seems to be distinct evidence of individual differences in the power of resistance against the ill effects of that deficiency. The rations used evidently contained small quantities of the vitamin; and on that small margin some of the animals were able to continue growing, though at a reduced rate. In the groups of very young or of half-grown animals, at least, this could not have been due to avoidable differences in inheritance, because in each case those that survived belonged to the same litters as those that did not. In this connection it may be noted that but one of our animals (rat 42) showed the paralysis symptomatic of polyneuritis.

It may be significant that four of the younger animals that had just enough resistance to maintain themselves on the rations were able to gain on the same ration after a period of about 100 days.

VITAMIN A TESTS. As a ration lacking in the fat-soluble vitamin a mixture of ground white corn and tankage, in the proportions of 85 to 15, was used. Such a mixture has been shown repeatedly to produce in rats the symptoms of lack of that vitamin. From the nature of the ration it cannot be said that it may not have some other deficiency besides that of vitamin A, but nothing else has been proved against it, and any other deficiencies which are known to exist in the white corn are surely abundantly supplied by tankage.

Six pairs of rats in all were used in the vitamin A test. Two pairs were of one litter, and four pairs were of another litter. The rats were started on experiment about as soon as they were ready to be weaned and the exercise was begun at once. They weighed at the start from

about 40 to 60 grams and were kept under observation on the same diet as long as they lived. Within each pair the difference in initial weight did not exceed 5 grams. The results on growth are presented in the accompanying chart in the form of curves based on the weekly averages of weight. The chart also shows the weekly food intake expressed as grams per 100 grams live weight.

The rats receiving the ration lacking vitamin A lived from 3 to 5 months. A period of good or moderate growth was followed by a period of approximately constant weight, with usually a very pronounced and rapid decline before death. The appetite usually remained good and the animals markedly active until they became quite weak. The symptoms, which gradually developed, were roughness of the coat and soreness in the eyes and sometimes the nose. None of the rats failed to develop these symptoms. The eyes first became dull and somewhat sunken, then the lids became swollen and pink at the edges and more and more closed, until finally a purulent and bloody condition kept them tightly closed for nearly all or all of the time. In some cases there was a distinct improvement for a time followed by a return of the severe condition later. The point most sharply marked in the development of the eye disease is the time when first there is evident difficulty in opening the eyes after sleep, the lids sometimes showing dried blood. The approximate time elapsing before such an eye condition was noted for the exercised and unexercised rat, respectively, of each pair was as follows: for rats 25 and 26, 13 and 14.5 weeks; for rats 27 and 28, 14.5 and 15 weeks; for rats 29 and 30, 11 and 12.5 weeks; for rats 31 and 32, 13 and 14.5 weeks; for rats 33 and 34, 10.5 and 14.5 weeks; and for rats 35 and 36, 10.5 and 12.5 weeks. It will be remembered that odd numbers refer to exercised rats and even numbers to unexercised rats.

The periods of survival of the individuals of the several pairs of these rats were as follows: rats 25 and 26, 106 and 124 days; rats 27 and 28, 127 and 144 days; rats 29 and 30, 108 and 132 days; rats 31 and 32, 109 and 142 days; rats 33 and 34, 89 and 119 days; and rats 35 and 36, 109 and 111 days. The weights at death were: of rats 25 and 26, 109 and 116 grams; of rats 27 and 28, 93 and 98 grams; of rats 29 and 30, 75 and 72 grams; of rats 31 and 32, 69 and 87 grams; of rats 33 and 34, 79 and 100 grams; and of rats 35 and 36, 75 and 98 grams.

It was noticeable that at all stages the pathological symptoms appeared in the exercised rat of each pair before they did in the unexercised rat, and that in each pair the exercised rat died before the unexercised. In all the pairs, except possibly one, the exercised rat declined more

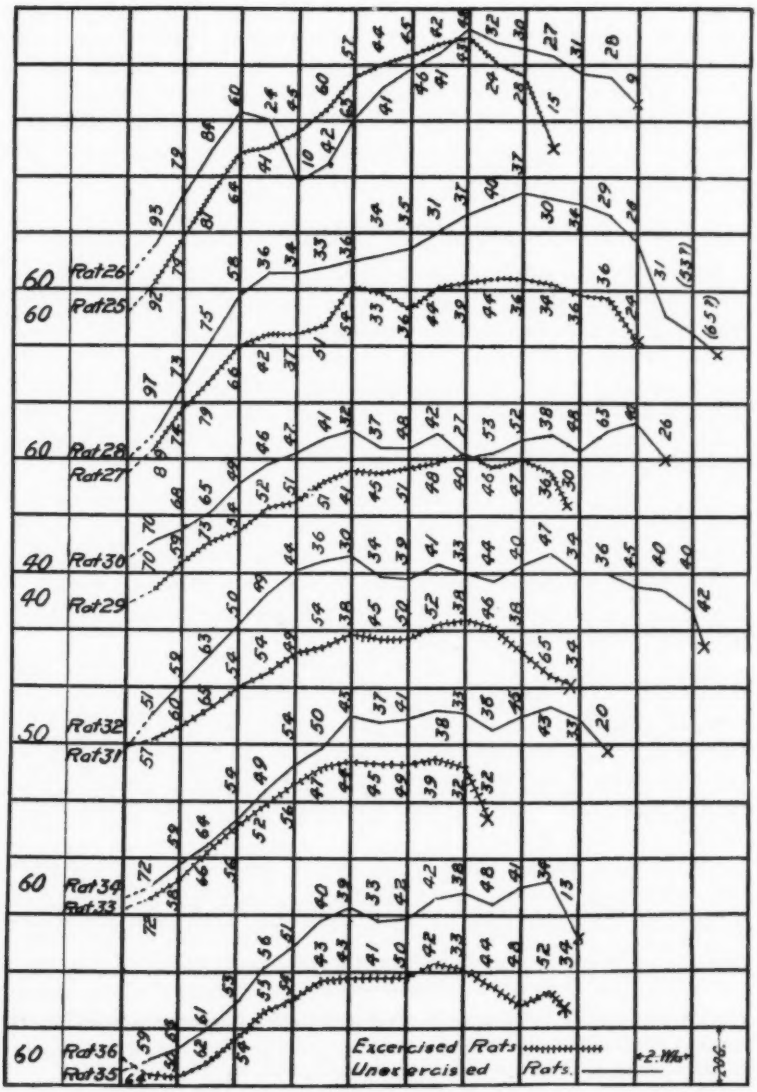

Fig. 1. Very young rats on a ration lacking vitamin A. The growth curves are derived from weekly averages of weight; the figures on the curves give the weekly food intake expressed as grams per hundred grams body weight. All the rats died from deficiency disease. In each pair the exercised rat died first; the exercised rats also all showed the disease symptoms before the unexercised.

rapidly after the eye trouble became pronounced than did the unexercised. These facts, which were quite evident in observation, are also clearly brought out by the data quoted above and by the curves shown in the chart. It can hardly be doubted that the exercise, or some condition contingent upon the exercise as applied, made the rats more susceptible to the ill effects of the lack of vitamin A. We attribute it to the exercise.

SUMMARY AND CONCLUSIONS

Rats have been employed in a test of the effect of muscular work on vitamin requirements. The effect of the muscular work was first tried with rats on complete rations and shown not to affect the growth curves in any consistent manner. Then the same test was applied to rats on rations deficient in vitamin B and to others on a ration deficient in vitamin A. In each case, one rat of a pair was made to exercise and the other not, while the two were fed comparable amounts of the ration. Weights and symptoms are reported.

The results indicate that the amount of exercise employed did not uniformly affect the well-being of the rats on complete rations or of those on rations lacking in vitamin B. They indicate that the exercise did distinctly hasten the appearance of the symptoms of vitamin A deficiency and hasten the death of young animals receiving insufficient amounts of this vitamin.

As regards vitamin B, therefore, no clear evidence was obtained that the amount and type of muscular work imposed on the rats in this test affected their requirement for this dietary factor. Possibly with rations still more deficient in vitamin B, positive evidence of such an effect would have been obtained, though in preparing the rations actually used in this test, it was expected that only insignificant traces of the vitamin would be present.

On the other hand, clear evidence was obtained that muscular exercise increases the demand for vitamin A. Apparently vitamin A is involved in the metabolism incident to increased activity of the voluntary muscles.

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THE INHIBITION OF ERECTION BY DECEREBRATION[1]

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Received for publication February 27, 1923

The nervous mechanism of erection has engaged the attention of investigators almost from the initiation, by Charles Bell and Magendie, of the modern study of nervous functioning. Thus Marshall Hall in 1837 (1) asserted on the basis of clinical evidence that the act of erection may be mediated by the posterior portion of the spinal cord following cord transection, and hence falls in the category of reflexes. Brachet in 1839 (2) cited an experiment on a cat definitely confirmatory of Marshall Hall's assertion, although Brachet failed completely to realize the significance of his own observation. Various authors (3) including most recently Riddoch (4), have verified the original conclusion of Marshall Hall.

From time to time additional information respecting the nervous mechanism of erection has come to light. Thus we have the classical demonstration by Eckhard (5) of the erectile function of the pelvic nerve (N. erigens), and some scattered observations bearing on the influence, obvious from ordinary experience, which the anterior part of the central nervous system may have upon the erectile process. Among these are the finding by Budge (6) that erection and ejaculation may be induced in rabbits by electrical stimulation of the pedunculi cerebri, and the observations of A. Goetz (7) on the occurrence of the same phenomena in criminals during execution by hanging.

Since the work of Eckhard (loc. cit) it has been known that the motor innervation of the erectile mechanism is by means of a portion of that subdivision of the nervous system known modernly as the autonomic system. In recent years the functional analysis of the autonomic mechanism has been carried far, particularly by such observers as Cannon (8), with the result that we are able to form fairly satisfactory pictures of the interrelations between various affective states and autonomic

[1] A preliminary report of this investigation is given in the Proceedings of the American Physiological Society: this Journal, 1922, lix, 461.

manifestations which they accompany or on which they depend. In a paper shortly preceding and leading up to his main work on the subject Cannon (9) discusses the antagonism between that part of the autonomic system whose excitation induces erection, namely, the sacral division, and the part to which he assigns the function of mobilizing the bodily energies in time of stress, namely, the thoracico-lumbar division. Cannon shows that affective states characteristic of bodily stress give rise to or, if not themselves causative phenomena, are associated with central nervous manifestations which give rise to inhibitory influences by which erection-inducing nervous discharges over the sacral autonomic division are either suppressed or made of no effect.

We have to report in this paper the discovery of a procedure by which a disturbance confined, so far as its experimental features are concerned, to the brain, makes peripheral stimulation of the pelvic nerve (N. erigens) ineffective as a means of inducing erection.

EXPERIMENTAL. Our experiments were performed on dogs anesthetized, usually, with A. C. E. mixture following subcutaneous morphine sulphate; in a few cases ether was used following the morphia administration.

Under deep anesthesia an incision was made in the linea alba, exposing the bladder. The bladder was opened at its summit and a cannula inserted to allow ready drainage of urine. The pelvic nerve is approached by separating the fascia to bare the rectum dorsal to the prostate gland. The nerve is seen embedded in the fascia covering the rectum. Shielded electrodes were placed on the uncut nerve and stimulation was applied by means of an ordinary inductorium. A strength of stimulus of the order of magnitude of that just barely perceptible on the tip of the tongue was used. The penis was exposed by cutting the skin ventrally as far back as the scrotum, and after ligating points of bleeding was inserted in a plethysmographic tube far enough so that the entire organ beyond the bulb lay within it. The rubber cuff was made air-tight by layers of vaseline and loosely packed cotton soaked in vaseline. To avoid the possibility of the penis being withdrawn from the tube by movements of the body or by contraction of the retractor penis muscle, a ligature was passed through the rubber cuff of the plethysmograph and through the fibrous sheath of the organ. The plethysmograph tube was immobilized by a clamp fastened to the operating board. Blood pressure tracings were taken by means of a cannula inserted in the femoral artery, connected with a Hürthle manometer. The latter was calibrated during the course of each experiment by comparison with a

mercury manometer, and at every stimulation a direct measure of arterial pressure was obtained by connecting the mercury manometer momentarily with the arterial cannula.

In sixteen out of seventeen successive experiments stimulation of either the right or the left pelvic nerve for from 20 seconds to 1 minute

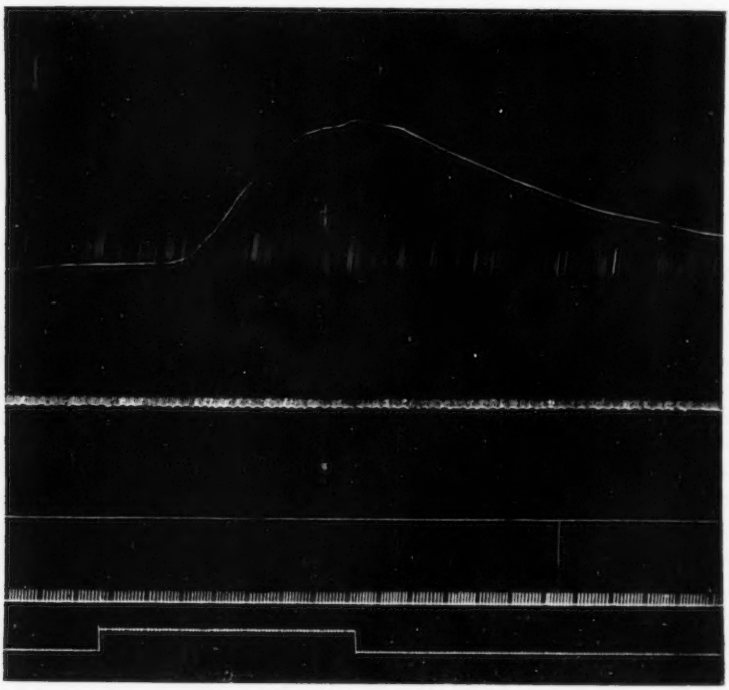

Fig. 1. Plethysmographic tracing of normal erection, induced by stimulation of the pelvic nerve. Arterial pressure 160 mm. Hg. Uppermost line, plethysmographic tracing. Lowest line, stimulation record; time in seconds.

was followed, after an average latent period of about 24 seconds, by normal erection (fig. 1). In several instances movements of the hind quarters and ejaculation also occurred. In a single case, no. 14, the distribution of nerve fibers in the fascia overlying the rectum was atypical, and the erectile fibers either were wanting or failed to be located.

In nine of the above experiments the animals, after the establishment

of normal erection, were decerebrated by vertical section through the brain stem at the level of the anterior limit of the tentorium cerebelli. The cut skirted the anterior limit of the cerebellum itself, passed between the superior and inferior colliculi, and traversed the crura cerebri inferiorly. All brain substance anterior to the cut was removed. Hemorrhage was controlled by tying of both carotid arteries and by manual compression of both vertebrals during and for a few seconds after the actual decerebration, if necessary. The blood pressure fell considerably, but in no case were the effects to be described due to low blood pressure, as we were at pains to show (see below).

In eight of these nine experiments stimulation of the uncut pelvic nerve gave wholly negative results, both immediately subsequent to decerebration and at intervals for as many hours afterward as the animals survived, in some cases three or more, there being no suggestion whatever of erection. In the ninth there was a slight engorgement of the penis but nothing approaching the normal erection that had been observed in the same animal previous to decerebration. In every one of these experiments we had positive evidence that the pelvic nerve was actually being stimulated adequately, since in every case strong unilateral contraction of the bladder was evoked by the stimulation. Since the strength and duration of stimulation were never less, but, on the contrary, usually greater after decerebration, when no erection was induced, than before decerebration, when normal erection followed stimulation regularly, and since, furthermore, the fibers within the stimulated nerve by which the bladder musculature was innervated were shown to be conveying impulses after decerebration as well as prior to it, we can scarcely escape the conclusion that the erectile fibers of the nerve were also carrying impulses in the former case as well as in the latter. This leads directly to the further conclusion that as the result of the decerebration some influence was set in play which made of no effect the nervous discharges over the erectile fibers.

The obvious influence which might have such an effect is the lowering of blood pressure attendant on the operation of decerebration. To test this possibility an experiment was performed on a non-decerebrate animal in which by successive hemorrhages the arterial pressure was lowered from an initial level of 150 mm. Hg to 30 mm. Hg. At pressures of 120, 90, 70, 50, 40 and 30 mm. erection followed stimulation of the pelvic nerve. The latency was about the same at each pressure level. With lowering of pressure the engorgement of the penis became progressively more gradual and less extensive, but there was never any

possible uncertainity as to the development of the response. Introduction of Ringer-Locke's solution by way of an external jugular vein raised the arterial pressure and restored the rapidity and extent of erection in precise correspondence. Immediately following these observations decerebration was performed, whereupon the erection reaction at once dropped out. The arterial pressure in this case was 30 mm. Hg. In our regular series of experiments in which erections were not obtained, pressures of 60, 70 and 80 mm. Hg were present after decerebration. This experiment would seem to rule out lowered arterial pressure as a means of accounting for the phenomenon.

In order to determine whether a general state of shock following the severe trauma might be responsible for the failure of erection, we performed four experiments in which the brain stem was purposely transected *anterior* to the level of the colliculi. In every case a slice of brain tissue at the level of transection was carefully removed for subsequent examination, which showed that these transections involved the third ventricle. In all these experiments erection, proportional to blood pressure, which in one case was as low as 22 mm. Hg, occurred upon stimulation of the pelvic nerve. In one of the experiments the brain stem was sectioned twice, both times anterior to the colliculi, and after both sectionings erections were secured. In another experiment a first sectioning was at the anterior level and was followed by erection, while a second sectioning, at the level of the colliculi, resulted immediately in a disappearance of the response (fig. 2). These experiments show that the inhibition of erection depends on transection at or near some particular structures and not on injury to brain tissue as such.

In view of the findings of former observers that erection may be induced in animals or men after transection of the lumbar cord, we desired to determine the effect of decerebration in combination with cord transection. Accordingly in an anesthetized animal in which stimulation of the pelvic nerve induced erection normally we transected the cord at the level of the second lumbar vertebra. After this transection erection was secured precisely as prior to it. Decerebration at the level of the colliculi did not in this case abolish erection, which was elicited in typical fashion by stimulating the pelvic nerve. In another experiment, the single one mentioned above in which possible slight erection was obtained after regular decerebration, subsequent transection of the cord at the level of the first lumbar vertebra was followed by a pronounced increase in the erectile response; this notwithstanding the fact that after cord transection the arterial pressure was only 30 mm.

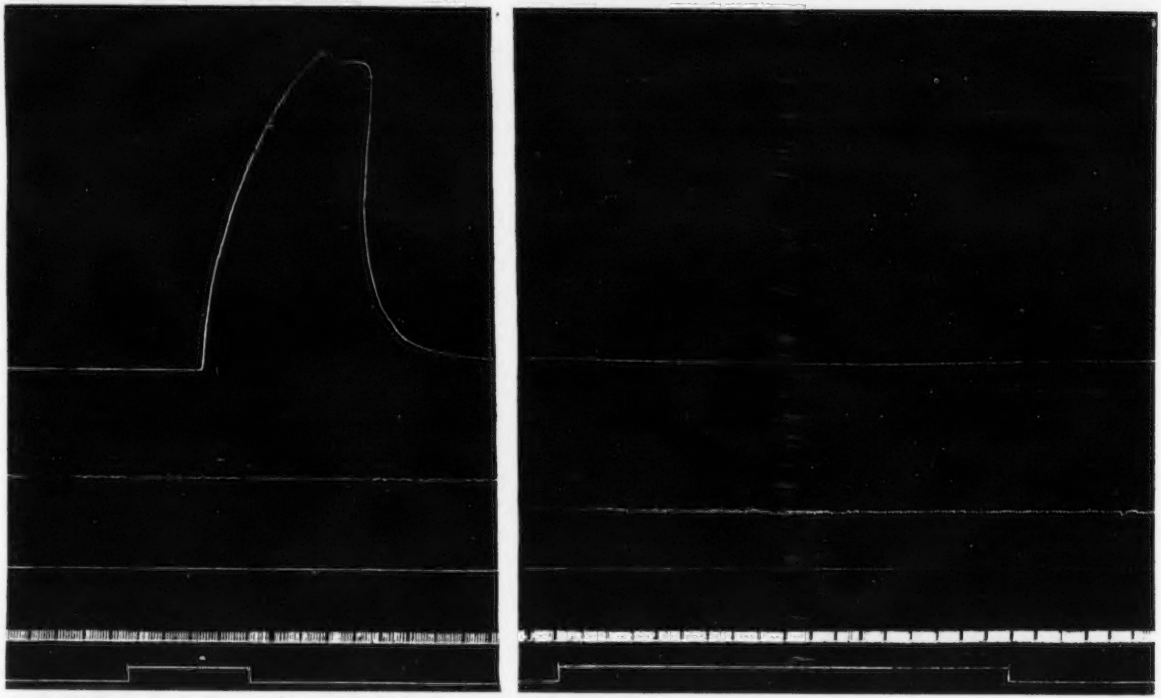

Fig. 2. Marked erection evoked by stimulation of the pelvic nerve after brain transection at the level of the third ventricle (left-hand curve), contrasted with entire absence of erection in the same animal upon similar stimulation after brain transection at the level of the colliculi (right-hand curve). Marked contraction of the bladder occurred upon each stimulation. Meaning of lines same as in figure 1.

Hg, as compared with 70 mm. Hg before cord transection, and subsequent to decerebration (fig. 3).

In all the experiments thus far described the pelvic nerves were intact, stimulation being applied to them by means of the shielded electrodes of Ludwig. This circumstance leaves open the question as to whether the pathway by which the decerebration inhibition becomes effective is over the pelvic nerves or by some other channel. To gain information on this point we performed an experiment in which both pelvic nerves were cut prior to decerebration. Normal erection occurred when either cut nerve was stimulated peripherally and it was as pronounced after decerebration as before. This experiment, taken in connection with those described just previously, in which erection was secured in

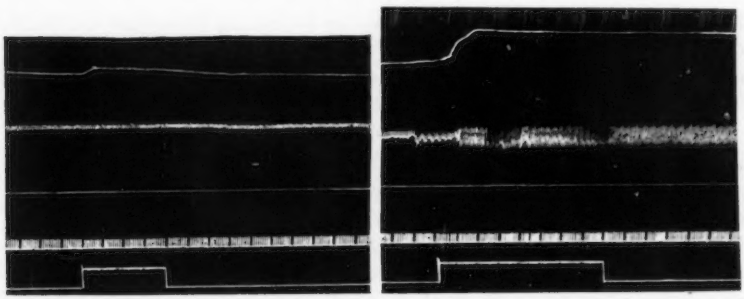

Fig. 3. Left-hand tracing, the very slight erection observed in a single animal after brain transection at the level of the colliculi; arterial pressure, 70 mm. Hg. Right-hand tracing, the pronounced erection obtained in the same animal after transecting the lumbar cord; arterial pressure, 30 mm. Hg.

decerebrate animals in which the lumbar cord was transected, would seem to indicate the pathway of the inhibition to be down the spinal cord from the brain stem and along the pelvic nerves. However, this simple interpretation encountered a difficulty when, in two subsequent experiments, we attempted to restore the response of erection by cutting the pelvic nerves *after* the inhibition had been established by decerebration. In neither experiment was erection thus restored, although in both we had positive evidence afforded by strong contractions of the bladder musculature that the pelvic nerves were functioning. In both experiments extreme care was taken that no aberrant twigs of the pelvic nerves should escape sectioning. Long stretches of fascia were destroyed in either direction from the nerves themselves to insure

against the possibility of nerve branches too minute to be seen being left intact. In these experiments we cut the hypogastric nerves as well as the pelvic, and in one of them we transected the cord in the lumbar region, but without obtaining restoration of the erectile response. The arterial pressure remained above 40 mm. Hg throughout the one of these experiments in which the cord was transected; it fell somewhat lower in the course of the other one.

In a final effort to locate a nervous pathway over which the inhibition might function, we performed an experiment in which we cut both pudic nerves after the abolition of erection by decerebration. There was no restoration of the erectile response following this procedure. The arterial pressure did not fall below 60 mm. Hg.

Discussion. Our evidence seems to show clearly that by sectioning the brain stem at the level of the colliculi in the dog influences are set in operation which act peripherally on the erectile structures of the pelvis, and in such fashion as to prevent them from responding to direct stimulation over their motor nerves. This implies some path of communication between the brain stem and the pelvic region over which the inhibitory influence can travel. That the influence is purely nervous seems to be negatived by our repeated failure to secure restoration of the erectile response by the cutting of all the nervous connections after the inhibition has become effective. On the other hand, the fact that if these connections are severed prior to decerebration no inhibition occurs shows that nervous continuity between the regions is essential for establishing the inhibition. An obvious suggestion by way of explanation is that the sectioning of the brain stem at the level of the colliculi gives rise to a stream of nervous discharges down the cord and over the pelvic nerves the effect of which is to induce a persistent inhibitory state. It is conceivable that an endocrine mechanism may be involved. If such a mechanism were present and susceptible to stimulation from brain centers not only would the phenomena described in this paper be accounted for but also certain familiar clinical situations. This suggestion assumes an endocrine gland whose secretion inhibits erection. It is in line with the general fact that persistent effects in the body are usually maintained chemically. Further experimentation is necessary before a definite conclusion in regard to this possibility can be reached.

SUMMARY

1. The erection which is readily evoked by stimulation of the pelvic nerve (N. erigens) is inhibited in dogs by transection of the brain stem at the level of the colliculi.

2. The inhibition is not due to lowered arterial pressure, nor to experimental brain trauma as such, but is specific for brain transection at the level indicated.

3. Establishment of the inhibition appears to depend on the integrity of the nervous pathways between the brain and the pelvis, but when once established the inhibition persists after their interruption, suggesting the intervention of an endocrine mechanism.

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THE EFFECT ON THE METABOLISM OF BREATHING PURE OXYGEN AT A PRESSURE OF ONE ATMOSPHERE

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Received for publication March 3, 1923

This paper presents the results of a preliminary study of the metabolism of canaries, exposed for short periods to pure oxygen at a pressure of one atmosphere.

The experiments were carried out by means of a special apparatus, constituting a closed system and so designed as to enable one to read directly the volume of oxygen consumed by the bird; the true volume being derived by the application of a few simple corrections occasioned by changes in temperature, barometric pressure and the solubility of gases.

THE APPARATUS AND METHOD. The apparatus consists essentially of two balloon flasks, each having a capacity of 2200 cc. a small animal chamber of cylindrical form, 2 inches in diameter and 3 inches in length, a by-pass and two 50 cc. burettes, arranged in manometer fashion and connected at the bottom by rubber tubing, thus permitting movement up and down. (See fig. 1.)

The two flasks together contain 3400 cc. of a 0.5 per cent solution of potassium hydroxid, leaving an air space in the two flasks of 1000 cc. By alternately raising and lowering the flasks, the solution siphons from one to the other and causes the air to move to and fro through the system. The neck of each flask is wound with asbestos cord to prevent undue temperature change during manipulation. All glass tubing, rubber connections and stop-cocks have a bore of a quarter of an inch, which is sufficiently large to permit a flow of 1000 cc. of air in 1 to $1\frac{1}{2}$ minutes.

During the preliminary period and the period immediately following the experiment proper, the animal chamber is shut off from the circuit and the air is made to flow through the stop-cocks *14* and *15* of the by-pass. In the experimental period, the air is directed through the animal

chamber *3*, instead of the by-pass. The carbon dioxid, produced by the animal, is carried into the flasks and removed by the caustic potash. The meniscus in burette *4* rises, owing to the contraction in volume. By bringing the meniscus in burette *5* to the same level as the meniscus in burette *4*, atmospheric pressure is restored. Burette *4* is always read with system at atmospheric pressure. The difference between the original and final readings of burette *4* represents the contraction in volume. To obtain the actual consumption of oxygen the contraction in volume must be corrected. The burettes contain glycerinated dis-

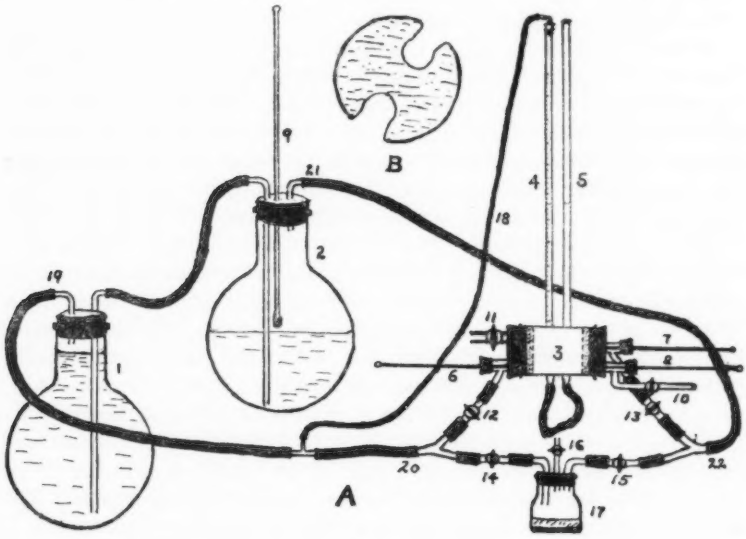

Fig. 1

tilled water. The addition of a few drops of glycerin has been found useful in preventing a distorted meniscus.

In order that the animal may not interfere with the free movement of air through the chamber, the openings into the chamber are protected with a circular piece of wire gauze, a perforated sheet of cork intervening between the gauze and rubber stopper. The manner of perforating the cork is illustrated in figure 1, *B*.

The rubber tubing between the flasks and by-pass has a wall of $\frac{3}{16}$ inch to prevent kinking and change of volume during manipulation.

The rubber tubing connecting burette *4* with the system has a fairly heavy wall and a small internal diameter.

Thermometers *6*, *7*, *8* and *9* are provided for taking the temperatures of the air and solution in the flasks and temperature of the air as it leaves the animal chamber. The chamber thermometers are graduated in degrees centigrade and their bulbs are concealed to avoid contact with the animal; the flask thermometer is graduated in tenths of a degree centigrade, the bulb being 1 inch above the surface of the solution when the flasks are in the position indicated in the figure. The glass tubing containing the chamber thermometers has an internal diameter of $\frac{1}{2}$ inch and a negligible dead space.

Procedure. Having tested the apparatus for leaks, which is easily done by closing the system and raising or lowering burette *5*, the animal is placed in the chamber at least a half an hour before the beginning of the actual experiment. The necessary ventilation is secured by aspirating air from the room through the chamber by means of a filter pump or Cenco blower, which is connected with the apparatus at *10*. The room air is first passed through a small wash bottle containing a little water and attached at *11*. The reason for this will appear later.

Meanwhile, the flasks are raised and lowered and gently shaken, the air during this preliminary period being directed through the by-pass. A record is now begun of the barometric pressure and the temperatures of the air and solution in the flasks. When the flask containing the thermometer is in the raised position, the air temperature is recorded and when the flask is in the lowered position the solution temperature is recorded. These manipulations and observations are continued until all changes in volume as indicated by the burettes can be accounted for by changes in the barometric pressure and flask temperatures. The volume changes are obtained with system at atmospheric pressure.

Coincidently with the above observations, it is necessary also to record the temperature of the air previous to its entrance into the chamber, by means of a thermometer in the wash bottle, and the temperature of the air as it leaves the animal chamber, registered by thermometer *8*. The average of these temperatures is taken as the true chamber temperature. If there has been no change in room temperature and no noteworthy movement on the part of the animal, thermometer *8* will reach a constant point in about 5 minutes.

The actual experiment begins with the closing of stop-cock *10* followed by the closing of stop-cock *11*. This order is important as it avoids a negative pressure in the chamber due to aspiration of air by the pump.

The air is now made to flow through the animal chamber instead of the by-pass, by opening stop-cocks *12* and *13* and closing stop-cocks *14* and *15*. This change from by-pass to animal chamber has always been made with the air of the system in motion.

Note. In the beginning a slightly different procedure was employed at this point which necessitated the introduction of a bottle in the by-pass. The procedure was abandoned, but the bottle was retained, because the small stop-cock *16* was found conveniently situated for the opening of the system and the readjustment of the menisci at the beginning of the next experiment. The bottle contains a small quantity of a one-half per cent solution of caustic potash.

During the experiment proper, the flasks are raised and lowered and gently shaken, but the barometric pressure and flask temperatures need not be recorded. A record is simply made of the temperature of the air leaving the animal chamber and registered by thermometer *6* or *7*, depending on the direction of air movement. The experimental period must never be concluded until these thermometers have become constant. The true chamber temperature during this period is found by averaging the flask air temperature, registered by thermometer *9*, and the temperature of the air as it leaves the chamber. The correctness of this procedure is attested by the fact that this average is, in general, almost identical with the true chamber temperature during the preliminary period, provided the rate of ventilation has been the same during the two periods. The reason that the average of the readings of thermometers *6* and *7* cannot be taken as the true chamber temperature, may, at first sight, not be quite so clear. During the preliminary period the mercury in thermometers *6* and *7* has risen considerably because of their proximity to the warm air of the chamber. They, therefore, indicate the temperature of the air leaving the chamber, very quickly, once the experiment proper begins. But the flask air does not assume the temperature of the air leaving the chamber, because most of the heat is lost to the room and the solution. Consequently, the difference between the two temperatures is too great to enable the chamber thermometers, because of their lag, to accurately register the temperature of the air entering the chamber in the time that is required for 1000 cc. of air to pass through the chamber. Since the temperature of the flask air increases but very little in the experimental period, it suffices to use the last record of the flask air temperature in the preliminary period, in arriving at the true chamber temperature.

At the close of the experimental period and while the air in the system is still moving, the chamber is shut off from the circuit by first opening

stop-cocks *14* and *15* and then closing stop-cocks *12* and *13*. The chamber is now opened at the points *10* and *11* and ventilation by aspiration renewed. But before making the final observation of oxygen consumption, it is necessary to continue raising and lowering the flasks so that any residual carbon dioxid may be absorbed. Meanwhile, the barometric pressure and the temperatures of the flask air and solution are recorded just as in the preliminary period, until equilibrium is restored.

The difference between the reading of the burette with the system at atmospheric pressure and the last reading of the burette in the preliminary period with the system at atmospheric pressure, is due to the consumption of oxygen on the part of the animal and to certain changes in temperature, barometric pressure and the solubility of gases that have taken place during the procedure. In general, therefore, corrections will need to be applied in order to obtain the actual consumption of oxygen.

Corrections. It is desirable for accurate work that the experiments be conducted in a room of fairly constant temperature. If the temperature is steadily rising or falling, the lag of the thermometer introduces a variable error. Under favorable conditions, the change in the temperature of the flask air seldom exceeds 0.1 to 0.2°C. during a determination.

The manner of finding the temperature of the animal chamber, in both the preliminary and experimental periods, has already been explained. If the chamber thermometers were allowed to become constant and the rate of ventilation, in the two periods, was the same, the change in the temperature of the chamber during the experimental period will scarcely ever be more than 0.3 to 0.4°C. and frequently the temperature at the end of the two periods will be identical. A given temperature change in the chamber air gives rise to a volume change of about one-tenth of that caused by an equal temperature change in the flask air, because the volume of the chamber is approximately one-tenth of the flask air.

Occasionally, the barometer remains steady during a determination, but, in general, there will be slight fluctuations in pressure and with the air of a barometer, reading to hundredths of a millimeter, any increase or decrease in volume due to barometric changes can be determined with considerable accuracy.

An additional correction will be necessary for alteration in the solubility of gases arising from change in the temperature of the solution and the percentage composition of the air in the system. An increase in temperature diminishes the volume of gas in solution and consump-

tion of oxygen on the part of the animal and removal of carbon dioxid by absorption reduce the tension of oxygen and increase the tension of nitrogen in solution. But the volume of oxygen liberated from the solution is somewhat in excess of the extra volume of nitrogen taken up, so that the volume of the air in the system is actually increased.

In order to avoid important changes in vapor tension, the precaution is taken of passing the air used for ventilation in the preliminary period through a small wash bottle containing a little water. To insure complete saturation, moist muslin is fastened on each piece of wire gauze in the chamber. If the chamber air were not completely saturated in the preliminary period, it would take up water during its passage through the flask, in the experimental period, with an increase in its vapor tension, whereas air entering the chamber from the flask would not necessarily undergo any change in its vapor tension. There would thus be a net increase in the vapor tension of the chamber with a corresponding increase in the total volume. For example, if chamber air at 24°C. having a relative humidity of 30 per cent and a vapor pressure of 6.0 mm. Hg enters the flask at a temperature of 21°C., where it comes in contact with the solution and becomes saturated, the vapor pressure increases to 18.5 mm.; but, flask air, already saturated at 21° and having a vapor tension of 18.5 mm. will, upon taking the place of the original chamber air, still exert a vapor pressure of approximately 18.5 mm. at a temperature of 24°C. The validity of this reasoning can be proved by experiment. It is assumed that the dilute solution of potash exerts the same vapor tension as pure water, which, of course, is not strictly true, but the error involved is negligible.

The actual corrections employed, calculated on the assumption that the flask contains 1000 cc. of air and 3400 cc. of solution and that the chamber and bottle in the by-pass each contain 100 cc. of air, are as follows:

1. A rise or fall of 0.1°C. in the temperature of the flask produces an increase or decrease of 0.4 cc. in the volume of the system.

2. A rise or fall of 0.1°C. in the solution temperature produces an increase or decrease of approximately 0.1 cc. in the volume of the system.

3. A rise or fall of 1°C. in the chamber temperature produces an increase or decrease of 0.4 cc. in the volume of the system.

4. A rise or fall of 0.3 mm. Hg in the barometer produces a decrease or increase of 0.4 cc. in the volume of the system.

5. The error arising from change in the solubility of gases due to consumption of oxygen by the animal will depend on the volume consumed and the temperature of the solution, as shown in the following table.

SOLUTION TEMPERATURE	TOTAL OXYGEN CONSUMED	CORRECTION (+)
°C.	cc.	
20	10	0.4
30	10	0.3
20	15	0.6
30	15	0.5

Check experiments. The method can easily be tested for accuracy. A small flask of water at about 50°C. is placed in the animal chamber. The chamber is ventilated with air saturated with water vapor and the usual preliminary observations are made. A carefully measured volume of air is removed from the system immediately after the beginning of the experimental period. The flasks are raised and lowered and the necessary observations recorded for 5 to 10 minutes, whereupon the experiment is brought to a close. A few final manipulations and observations having been made to determine whether the system is in equilibrium, the contraction in volume is calculated. It has been found that the corrected volume agrees remarkably well with the volume actually removed (table 1). In these experiments all the corrections were made with the exception of those for changes in the solubility of gases due to change in the composition of the air.

Experimental results. A few experiments were made for the purpose of finding out what influence food would have on the results. The question of variety of food does not enter, because it is always of the same kind, namely, a mixture of canary and rape seed; nor is it of importance to know the interval that elapsed between the last feeding and removal from the cage, because a canary feeds frequently the greater part of the day. Experiments indicated that, if the experiments were begun from 30 minutes to an hour after removal from the cage, the first determinations of a series were sometimes higher and sometimes lower than those that followed and the variation in the results was no greater than one might expect in a fasting animal. This was true only if the bird was accustomed to the chamber. The element of fear or anxiety is probably of greater importance than food. With a little training, however, the canary will soon adapt itself to the restraint of the chamber.

TABLE 1

DATE	VOLUME ACTUALLY REMOVED	VOLUME FOUND
	cc.	cc.
11–8	11.3	11.5
11–8	11.5	11.4
11–13	11.1	11.1
11–30	11.7	11.6
11–30	5.9	5.9
12–1	12.7	12.9
12–1	12.2	12.1
12–5	12.7	12.8
Average.....	11.13	11.14

TABLE 2

AIR				OXYGEN			
Date	Time	Temperature	Oxygen consumed per min. per kilo. in cubic centimeters. (N.T.P.)	Date	Time	Temperature	Oxygen consumed per min. per kilo. in cubic centimeters. (N.T.P.)
Bird A							
		°C.				°C.	
11–4	12.47	23.0	96	11–18	12.56	20.0	102
11–6	12.45	21.0	100	11–20	1.14	21.0	107
11–8	1.19	22.5	97	11–21	2.13	21.0	110
11–11	9.47	21.0	106	11–21	2.51	21.5	106
11–11	11.08	22.0	104	11–22	2.20	21.5	90
11–13	2.36	22.0	95	11–23	1.14	23.0	93
11–13	3.15	23.5	96	11–23	1.55	23.5	92
11–15	3.26	23.0	89	11–23	2.24	23.5	92
11–17	3.01	21.5	100	11–23	3.27	24.0	92
11–29	2.50	22.0	105				
Average		22.2	99.0			22.1	98.2
Bird B							
11–14	1.38	21.0	109	11–20	3.26	21.5	89
11–14	2.10	22.0	103	11–21	12.56	20.0	110
11–15	12.50	23.0	88	11–21	1.28	21.0	102
11–15	1.30	23.5	92	11–22	3.30	22.0	93
11–17	1.30	21.0	97	11–22	4.10	22.0	89
11–17	2.14	22.0	98				
Average		22.1	97.8			21.3	96.6

It is extremely difficult to observe the respirations with any exactness throughout an entire experimental period. Frequent counts have shown that the particular species of canary used in this series of experiments has a respiratory rate, while in the chamber, between 100 and 140 per minute. The rate may be very much higher, if the canary is not habituated to the experiment. In the cage the respirations have been observed to vary from 90 to 120.

The experiments proper fall into two groups, those in which the apparatus was filled with atmospheric air and those in which it contained pure oxygen. The results of both groups are tabulated in table 2. The figures in the last column represent the resting metabolism of the bird. All experiments in which there was more than the slightest amount of movement were rejected. Both canaries were females and belonged to a variety having a comparatively dark plumage. In calculating the metabolism, the gross weight of the bird was used, no al owance being made for the weight of the feathers.

In the presence of air, bird A consumed, on the average, 98.9 cc. of oxygen per minute per kilogram of body weight. Bird B had a slightly lower metabolism and consumed, on the average, 97.5 cc. of oxygen per minute per kilogram. The normal resting metabolism of the canary may therefore be taken as 98.2 ± 1.45. The flasks were always filled with fresh air at the beginning of each experiment.

For the purpose of ascertaining the influence on the metabolism of pure oxygen at one atmosphere of pressure, the flasks were filled with oxygen through an opening not shown in the figure. With the system closed, the flasks were shaken thoroughly until further shaking no longer caused a contraction in volume, as indicated by the burettes, allowance being made, of course, for change in temperature and barometric pressure. The flasks were then permitted to stand over night, the shaking being resumed on the following morning to insure complete saturation. Having produced perfect saturation, it was only necessary to add a little oxygen at the beginning of each experiment. The technical procedure in these experiments was precisely similar to the one used in the air experiments, except that no correction was required for change in the solubility of gases due to change in the composition of the gaseous content of the flasks, because the apparatus would always contain pure oxygen, no matter how much the bird might consume.

The canary breathed oxygen during a part of the preliminary period as well as during the actual experiment. So that while the experiment lasted 6 minutes, the bird was exposed to oxygen for a period of 15 to

30 minutes. The oxygen was aspirated from a Douglas bag through a wash bottle to saturate it with moisture and thence through the chamber.

It will be observed that in both instances the metabolism in the presence of oxygen was slightly lower than in the presence of air, the average of the two birds being 97.3 ± 2.06 cc. of oxygen per minute per kilogram. The result, however, cannot be interpreted as a real change in the metabolism, in view of the probable error of the difference between the means.

In experiments of this character, it is important to give careful attention to the matter of temperature. The canaries were exposed to temperatures varying from 20 to 24°C. This is not a considerable range and yet the influence of temperature on the metabolism is clearly indicated. The average consumption of oxygen at 20 to 21° was 105, at 21.5 to 22.5°, 97; and at 23 to 24°, 92 cc. of oxygen per minute per kilogram. It will be seen by reference to table 2 that the average temperature to which bird A was exposed was the same in both groups of experiments, whereas bird B was exposed to an average temperature which was 0.8° lower in the oxygen experiments than in the air experiments. If, in the case of bird B, the range of temperature had been exactly the same in the two sets of experiments, it is likely that the result for the metabolism in oxygen would have been even lower than the one actually obtained, but the difference between the mean for air and the mean for oxygen would still have remained insignificant.

In 1911, Benedict and Higgins (1) carried out a series of experiments with the view of determining the effect of the inhalation of oxygen on the metabolism of man. They concluded that, as indicated by the gaseous exchange, the breathing of mixtures containing 40, 60 and 90 per cent of oxygen does not alter the metabolism of normal individuals lying at complete muscular rest, 12 hours after the last meal. The experimental period was usually 10 to 15 minutes.

CONCLUSION

The experimental results justify the conclusion that when canaries are exposed to pure oxygen at a pressure of one atmosphere, for periods varying from 15 to 30 minutes, the metabolism, as determined by the method described in this paper, undergoes no demonstrable change.

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THE DIASTOLIC PHASES OF THE CARDIAC CYCLE IN MAN

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Received for publication March 5, 1923

Although it is well known that ventricular filling by no means takes place throughout diastole, it is not commonly emphasized that blood does not flow into the ventricle until the process of muscular relaxation has practically been completed. In other words, the rate and duration of muscular relaxation are in no wise related to the rate and duration of ventricular filling.

To emphasize these relations and to distinguish them on records of dynamic events it has been found necessary to subdivide the period of diastole into a number of distinct and consecutive phases (Wiggers). At the onset of muscular relaxation the semilunar valves are in the act of closing, thus forming the first diastolic event, viz., the proto-diastolic phase. During the remainder of muscular relaxation both sets of valves remain closed and the ventricle relaxes isometrically (isometric relaxation phase). Under normal conditions of venous pressure, the A-V valves do not open and blood does not begin to enter the ventricle until this relaxation has been completed. Inasmuch as the process of relaxation is quite as fundamental an attribute of muscle tissue as that of contraction it is possible that the duration of the isometric relaxation phase may supply valuable information as to the condition of the heart muscle. The bulk of experimental evidence indicates that after the opening of the A-V valves, inflow at first occurs rapidly (rapid inflow phase) and as the intra-auricular and intraventricular pressures become equalized, proceeds more slowly (diastasis). This continues until the supervention of the next auricular systole which again increases the rate of filling.

It is obvious therefore that the length of the rapid inflow and diastasis phases are criteria of filling mechanisms in the heart and their determination becomes important, because if the dynamic laws obtained from experimental work on dogs are to be transferred to the human

heart it is necessary to be quite certain that the apportionment of these diastolic phases corresponds reasonably well. For these reasons a study of the duration of the diastolic phases in man was undertaken, at the suggestion of Prof. C. J. Wiggers.

PROCEDURE. The separate phases of diastole in man can be determined with a fair degree of accuracy from the diastolic waves of the venous pulse provided this is recorded from a place low down in the neck, by optical apparatus. For this reason synchronous optical tracings of the arterial and supraclavicular venous pulses were recorded by segment capsules from 50 medical students and laboratory assist-

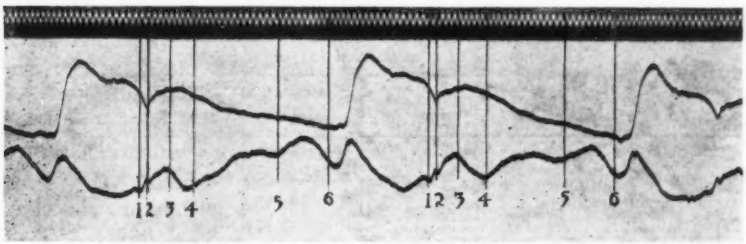

Fig. 1. Optical records of carotid and supraclavicular venous pulses in man, showing method of computing diastolic phases. *1–2*, protodiastolic phase; *2–3* isometric relaxation phase; *3–4*, rapid inflow phase; *4–5*, diastasis phase; *5–6*, probable duration of auricular systole. Time in 0.02 second. (Two-fifths actual size.)

ants between the ages 19 and 34. Of these 7 were women. All records were taken in the lying position.

A sample record is shown in figure 1, and on it are indicated the points selected as demarcating the successive phases. The chief error in determining the precise duration of the phases results from the fact that occasionally waves do not come to a sharp crest. In order to avoid as far as possible the personal element in such cases, only records with clear cut-waves were measured, the others being discarded.

RESULTS. The results of 187 carefully calculated pulse beats selected from the records of 50 individuals were first tabulated in detail. Owing to their length these tables are not published[1] but the results are concisely summarized in table 1.

[1] A photostat copy of the original tables can be obtained by application to Department of Physiology, W. R. U. Medical School.

When such extreme ranges occur as are shown in the first column of this summary the average durations become of little statistical value unless interpreted in the light of individual variations brought out in the original table.

In regard to the protodiastolic phase it was found that in over 50 per cent of cases the range lay between 0.035 and 0.045 second while 83 per cent of cases came within the ranges of 0.03 to 0.05 second. We may therefore conclude that 0.04 second roughly represents the average duration of this phase in man.

In regard to the isometric relaxation phase about 40 per cent of measured cycles showed durations within the ranges of 0.07 to 0.085 second

TABLE 1

Summary of results from 187 pulse beats

PHASE	WAVES COMPLETE RANGE	PREDOMINANT RANGE	ACTUAL AVERAGE DURATION
	second	*second*	*second*
Protodiastole	0.016–0.060	0.03–0.05	0.038
Isometric relaxation	0.037–0.130	0.06–0.09	0.076
Rapid inflow	0.055–0.173	0.09–0.13	0.113
Diastasis	0.08 –0.708		

TABLE 2

PHASE	AVERAGE	
	Man	Dog
Protodiastole	0.038	0.022
Isometric relaxation	0.076	0.048
Rapid inflow	0.113	0.045

and 70 per cent between the ranges 0.06 to 0.09. It would therefore seem that 0.076 second represents also a fair average of the predominant cycles.

As regards the phase of rapid inflow there is a greater variation in different individuals and consequently any average has less significance. Nevertheless it is interesting to note that in over 66 per cent of the cycles this phase varied 0.09 to 0.13 second. Still greater variations occur of course in diastasis and a plot of the results showed clearly that this phase lengthens proportionately to the duration of entire diastole. This, it may be added, is in striking contrast to all other diastolic phases which showed no correspondence whatsoever.

In table 2 a comparison is made between the average duration as found in these experiments and those recently reported by Wiggers in closed chest experiments on dogs.

It is obvious at a glance that all of these average phases are appreciably longer in man than in anesthetized dogs.

CONCLUSIONS

1. The importance of calculating the consecutive phases of diastole in man lies in the fact that in this way the duration of the inherent relaxation process (occurring during the protodiastolic and isometric relaxation phases) may be studied independently of the filling process (occurring during the rapid inflow and diastasis phases).

2. The definite suggestion develops that the duration of the isometric relaxation phase may prove to be a direct criterion of the condition of the ventricular muscle.

3. It is possible to calculate the duration of the successive diastolic phases in man by the use of the optically recorded venous pulse.

4. When both individual and average figures are compared, the relaxation time is definitely longer in man than in anesthetized dogs, and therefore a longer interval elapses after the onset of relaxation before the ventricle begins to fill from the auricles. The succeeding period of rapid inflow also continues for a longer time, consequently at corresponding heart rates the interval of diastasis in man must be shorter than in anesthetized dogs.

LESIONS OF THE CORPUS STRIATUM BY RADIUM EMANATION AND THE ACCOMPANYING STRUCTURAL AND FUNCTIONAL CHANGES[1]

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Received for publication March 5, 1923

The exact functional rôle of the group of nerve cells comprising the corpus striatum has presented one of the most difficult problems of neuro-physiology. In a large measure, the function of this part has remained hidden because its obscure anatomical position has rendered the usual methods of physiological investigation practically useless. A great variety of activities has been assigned to the striato body of the encephalon by earlier writers, but only within the past ten years has definite progress been made in the way of elucidating its physiology.

An excellent review of the literature relating to the studies on the corpus striatum has been given recently by Kennier Wilson (1), so it will not be necessary to repeat it here; suffice it to say that the basic idea upon which the modern doctrine of a motor regulatory function of this part is founded is an old one. The suggestion is made by Nothnagel (2) that the corpus striatum represents a sort of synthesizing center for involuntary movements, and Ferrier (3) expressed the view that an integrating center is located in this part for movements that are separately represented in the cerebral cortex. According to Prus (4), the corpus striatum exercises a kind of control over the tonic activity of the muscles.

In the more recent investigations the clinico-pathological method has yielded significant results. The studies of Wilson (5) and of Hunt (6) present notable additions to the subject of the functional rôle of the corpus striatum as a whole and of that of its different parts. A critique of the clinico-pathological method would be out of place here, but it is important to note, in this connection, that the basis for pathological

[1] A preliminary report is found in the Proceedings of the Society for Experimental Biology and Medicine, 1922, xix, 382.

changes in the corpus striatum has not been definitely determined. With the etiology unknown, it is impossible to assert to what extent the manifestations of a given pathological process may have been modified by other disturbances of a common origin but pathologically not so sharply defined. Likewise this method, since it depends upon a slowly progressing destructive process, with a corresponding change in function, does not give any idea of the degree of compensatory adjustment possessed by the organism.

Fournié (7) attempted to destroy definite portions of the corpus striatum by introducing zinc chloride, and Nothnagel (2) by the use of chromic acid similarly applied. They sought in this way to ablate certain portions of these ganglia and then to observe the functional defects. The diffusibility of these substances renders interpretations based upon such methods of questionable value. Different electrical stimulating devices have been assembled for the purpose of testing the corpus striatum function. The stimulating electrodes were so arranged as to permit of their being passed through brain structures without material damage. When properly directed, their stimulating tips were brought to act upon selected parts of the striato body. A summary of the results of this method shows various and conflicting points of view and great diversity of the experimental findings, but in general the method has failed to demonstrate an unmistakable motor control through this part elicitable by even very strong electric stimuli.

Of particular interest in this connection, however, is the work of Wilson (8) on the production in monkeys of electrolytic lesions from the passage of a constant current through selected areas of the corpus striatum. He used for the purpose of directing the electrodes the stereotaxic instrument devised by Horsley and Clarke (9). A careful study of the behavior of the animals so treated failed to show, in a period of about three weeks, any marked disturbances. At autopsy, distinct lesions were found, localized in either the caudate or the lenticular nucleus. Additional significance is attached to these results on the monkey because of its close neurological similarity to man and because of the well-founded conception that the functional importance of the corpus striatum bears an inverse relation to the phylogenetic order. It is interesting to note, also, that these experiments on the monkey, presenting a condition of uncomplicated striato degeneration, afford little support to the view of a motor-steadying function of the corpus striatum. It has been estimated (1) that in dogs the proportion of basal ganglia mass to the total brain volume is about 3 per cent

greater than in apes and about 6 per cent more than in man. If there exists in this part of the nervous system an intimate relation between the size of the structure and the magnitude of its physiological activity, then studies on the striatal part should yield in dogs slightly more marked results than in either of the other forms.

In the series of experiments to be described it was our aim experimentally to produce localized lesions of the corpus striatum of dogs by burying in this structure small tubes of radium emanation and to study the neuro-physiological reactions resulting from such destruction. Furthermore, the lesions were to be sterile and the operative procedure incident to the introduction of the tubes was to involve practically very little trauma. We would emphasize particularly that the method possesses outstanding advantages and is unique, so far as we are aware, in this kind of experimentation; that the experimental animals, being of a lower order in the developmental scale, should tend to exhibit more marked disturbances; and that the disturbances produced in this way are of necessity uncomplicated and afford full opportunity to observe the compensatory readjustments.

EXPERIMENTAL. *Methods and materials.* The experiments were conducted on dogs ranging in weights from 7 to 14 kgm. During the observational period they were kept in individual cages, and at frequent intervals their condition and motor responses were noted. The operative work was done under general morphine and local novocain anesthesia. A 2 mm. hole was drilled in the skull at about the intersection of the superior temporal line with the coronal suture, and through this hole a long, fine steel trocar was introduced. This trocar carries in its lumen a long plunger and when "loaded" contains at the pointed end a glass radium emanation tube. These tubes vary from about 2.5 to 3.5 mm. in length and are about 0.4 mm. in diameter, with walls about 0.1 mm. thick. They contain on the average 1 millicurie of radium emanation.

The method of depositing these tubes consisted of inserting the "loaded" fine trocar through the brain substance to such a position that its tip rested at the desired location, then the plunger was pressed, leaving the radium tube permanently embedded at the particular spot. The greatest difficulty obviously was presented in properly localizing the tip of the trocar, since we did not have at our command the precise stereotaxic device of Horsley and Clarke. After considerable practice with dead material, and by the aid of average measurements of several brains of dogs, we were able to accomplish localization with a fair

degree of accuracy. Some of the animals showed by the early development of spastic symptoms, and others by the autopsy findings, that the radium tubes had been wrongly placed. All of these instances were left out of consideration, and only those were accepted as pertinent to this study which showed at the autopsy lesions involving mainly the caudate or the lenticular nucleus.

The treatments were carried out in three ways, as regards time sequence. In some instances simple unilateral lesions were produced; in other instances animals having a unilateral lesion were treated, after a period of from two to three weeks, on the opposite side; and the third procedure was to treat both sides simultaneously. The animals were finally all brought to autopsy, the time at which this was performed ranging from two weeks to over seven months after the initial treatment. The brains were removed in all instances, and in many cases specimens were taken of the liver, spleen, kidney and other organs.

Nature of the lesion and the physical factors involved. It is well known that the beta-rays from radium emanation are easily absorbed by a few millimeters of tissue (10), so that when a small glass capillary tube, containing about 1 millicurie of radium emanation, is embedded in living tissues, definite localized destructive reactions occur, mainly due to the relatively intense beta-ray radiation given off in the immediate vicinity of the tube.

Bagg (11) has shown that normal brain tissue treated in this way and examined about two weeks later becomes completely necrotic in the immediate vicinity of the tube, while surrounding this area is a zone of polynuclear leucocytes beyond which there is some hyperemia. The entire lesion is limited to an area of 1 cm. in diameter. The lesion is sharply localized; the cells beyond the 1 cm. zone retain their normal morphological character. Control experiments have satisfactorily shown that the lesion is practically due entirely to the irradiation of the radium emanation and not to the presence of the small glass tube or the traumatism incident to its insertion.

The lesion just described is not produced at the same time the tube is inserted because, from previous data obtained by examining the radiated areas at different periods after the insertion, it was found that the lesion slowly increased in size. At the end of twenty-four hours the zone of necrosis about the tube was about 1 mm. wide, while the maximum of destruction (1 cm.) was reached at the end of about two weeks. Radium emanation loses its activity at the rate of 16 per cent each day, and in clinical usage the tubes, when used in this way, are said to have an effective period of irradiation equal to 132 hours.

RESULTS. *a. Histological.* The results may be divided into three parts: first, a microscopical study of the brain lesions; second, an examination of the sections of the spinal cord for degenerative fiber tracts; and third, an examination of the viscera, especially in the case of the liver following lenticular degeneration due to the experimental lesions.

A general description is given above of the type of brain lesion we are dealing with and its development from the time of insertion of the radium tube. The histological sections of two lesions are presented in this part as showing typical conditions. The first is taken from the

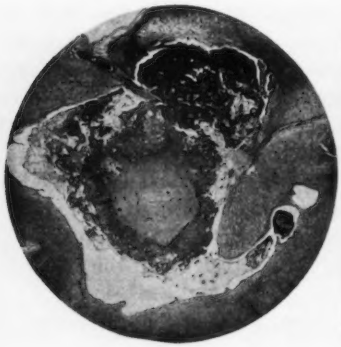

Fig. 1

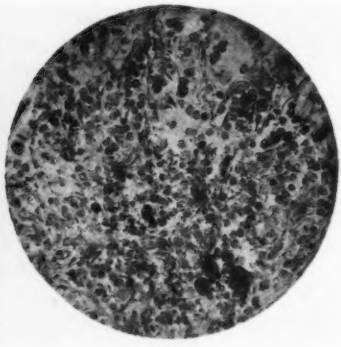

Fig. 2

Fig. 1. Low magnification of a striatal lesion showing a central area of complete necrosis, surrounded by a broad zone of fibrin. The surrounding brain tissue is apparently normal.

Fig. 2. High magnification of the central portion of the lesion shown in the gross in figure 4. Large pigmented Gluge's corpuscles are seen, but no neural elements are present.

right side of the brain of dog 11 (see fig. 1). In this case the lesion was about sixteen weeks old and resulted from the insertion of a tube of 1 millicurie placed in the anterior part of the striatum. Microscopical examination of the central portion of the lesion showed a necrotic area 4 mm. in diameter. Immediately surrounding this was a broad ring of fibrin and then an outside zone, 2 to 3 mm. in width, that exhibited edema and many Gluge's corpuscles, with a slight cellular exudate. The lesion was sharply demarcated from apparently normal surrounding tissue. The second histological section (see fig. 2) is shown in the gross

in figure 4. This lesion is of interest in that it represents a much older destruction than that in the experiment just described (actually thirty-three weeks old) and one that was associated with considerable shrinking of the caudate of that side. Microscopical examination shows that the lesion involves a considerable portion of the right caudate nucleus. A more detailed study of this lesion reveals an area of softening that is discolored by methemoglobin and is partly cystic. In the area of destruction there is a collection of large round cells, the Gluge's corpuscles. The latter are distended by fat droplets, yellowish granular pigment, and in places a dark substance that is evidently blood pigment. Similar Gluge's cells are found all along the side of the lateral ventricle, which is moderately dilated on this side of the brain from the atrophy of the caudate. No neural elements are to be found in this lesion. In the surrounding brain tissue there is apparently little alteration of structure.

The histological data of the brain lesions in this study have shown three typical conditions: first, a central necrosis sharply marked off by a ring of fibrin from the surrounding apparently normal tissue; second, an older condition in which reparative processes have begun, with numerous phagocytic Gluge's cells present; and third, a brain destruction represented by a central cavity filled with serous fluid and surrounded by fibrin and a zone of edematous tissue.

On the whole, the results have shown a pronounced localization of the lesion to a single focus in the brain. We have not found that circulatory disturbances are produced in brain tissue outside of the area of the experimental lesion as a result of cutting off the blood supplied by the vessels passing through the radiated area. Two factors are suggested as probably accounting in a large measure for this condition: First, there is the delay of a few days before the full development of the radium lesion takes place, and this permits of the reëstablishment of collateral circulation; and second, although in all of the instances studied the small blood vessels in the central portion of the lesion have been completely obliterated, we have observed cases in which the firmer coats of the larger vessels have apparently offered sufficient resistance to the destructive action of the radium rays to preserve physiological activity, even though the brain tissue around them has shown destructive changes.

A study of the cord sections taken from these animals has revealed no degenerative fiber tracts. No study has yet been made for degenerative tracts connecting the corpus striatum with other parts of the brain. It is planned to make this phase of the investigation the subject of a separate report.

The histological examination of the viscera revealed nothing that could be attributed as bearing any relation to the brain lesions. No hepatic cirrhosis was noted, which is usually associated with progressive lenticular degeneration in man.

b. Physiological. At the outset the attempt was made to limit the site of the lesion to the lenticular nucleus in some instances and to the caudate nucleus in others. This was found to be impossible on account of the comparatively small size of these structures in the dog. Fifteen dogs in all were treated, of which nine showed striato lesions of sufficient degree to warrant their inclusion in this study.

One animal out of the nine successful experiments was chloroformed for an autopsy at the end of two weeks following the introduction of the radium tube into its striatum. Sections of the brain of this animal revealed a lesion involving the anterior end of the caudate, the lenticular nuclei and some of the adjoining cortex. In this animal there had been no signs of motor disturbance nor any change in its behavior to distinguish it from a normal dog. It was the first animal treated in the series and the autopsy was performed early in order to throw light upon the nature of the lesion produced.

Of the remaining animals of the series seven were allowed to live for periods of from sixteen to thirty-three weeks, and the eighth one died at the end of four weeks, apparently of a pneumonia contracted during an operation the object of which was to insert a second radium tube on the opposite side of the brain. In five animals an initial treatment was given always on the right side, and then a second tube was inserted in the opposite side after an interval of from two to four weeks. With a single exception these dogs showed an immediate recovery from the operation and no unusual symptoms during the period of observation. The one exception to this rule presents some suggestive features. This dog, when first treated, was somewhat obese but apparently normal. The day following the operation there was nothing unusual in its behavior, but on the third day it exhibited a slight cough as if there was a mild irritation in the throat and, although the room was cool, it had the tongue out, with panting respiration. These symptoms continued for four or five days, but the general appearance of the animal during this time was good. By the eleventh day it appeared dull and moved about with head down. When fed on the fourteenth day a peculiar inability to chew and swallow was exhibited; meat was taken into the mouth but usually, after one or two chewing movements, the jaws ceased to act. A strong sensory stimulus seemed to arouse it and chewing and swallow-

ing would result, but perhaps the next morsel taken would bring forth a repetition of these acts. These symptoms gradually became less marked and by the twenty-second day the animal was again apparently normal. The brain of this dog, when sectioned, presented a lesion involving most of the right caudate and the anterior end of the lenticular. The temporary derangement in the swallowing mechanism, possibly a dysphagia, and the moderate disturbance in temperature regulation are interesting manifestations, but since lesions of a comparable character have been produced in a number of other dogs without the appearance of these signs, it is not warranted to attach especial significance to them.

In three animals a pair of radium tubes were simultaneously introduced, one into each hemisphere of the corpus striatum. These animals were kept under observation for sixteen, sixteen and twenty weeks, respectively, and at the end of these periods they appeared essentially normal. In the early part of the experimental period, however, they exhibited some neurological signs that are of interest. Some muscular unsteadiness in the form of a fine tremor appeared in all instances within a period of about four days after the tubes were introduced. This continued for about two weeks, with very little apparent change. A certain degree of clumsiness in the animals' movements was also noted, and in one instance there was shown a tendency to swaying of the posterior part of the body. One of these animals exhibited in a striking way a peculiar type of body pose, which was shown in some degree in several of the dogs in which the lesions were not produced simultaneously. This body attitude consisted of a marked rounding up of the back, a drawing under of the hind limbs and a spreading outward of the fore limbs, especially at the elbow joints. The condition is suggestive of a hypertonia and indicates, we believe, a disturbance in extra-pyramidal control. There were no signs of paralysis present nor indications of spasticity of the pyramidal type.

The striato lesions in these animals varied somewhat in degree, but were restricted for the most part to the anterior portion of the lenticular and the caudate nuclei and to some of the adjoining brain matter. Figure 3 sets forth in a characteristic way the type of lesion produced.

It should be noted in passing that in several of these animals with striato lesions there appeared within the first four or five days after the radium was introduced a sort of irritation of the eyes. One animal was observed a number of times to rub briskly the region of the eyes, and others showed some conjunctivitis and considerable mucopus-like discharge. The cause of this phenomenon is not clear, but it is suggested

that it may have been due to an indirect effect upon the optic thalamus, or perhaps to a direct action of the radium upon the optic tracts which were not, in most cases, very far from the site of the radium tubes.

It remains to add that in these experiments we have demonstrated that a surprisingly large destructive lesion and atrophy may be produced in the corpus striatum of the dog without significant changes in the animal's behavior. In figure 4 is shown a brain section in which there was destruction of the anterior part of the striatum with considerable shrinking of the caudate of that side. The early symptoms in this animal included tremors, some clumsiness in motor activity, irritation of the eyes, and for several weeks a distinct dulling and huski-

Fig. 3

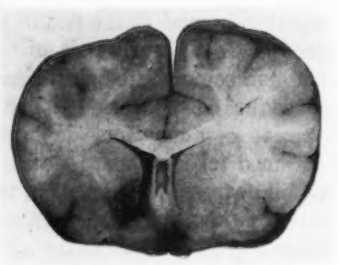

Fig. 4

Fig. 3. A bilateral lesion comprising the anterior portions of the caudate and lenticular nuclei. Two radium emanation tubes of 1 millicurie each were introduced sixteen weeks before this brain was removed.

Fig. 4. The atrophy of one caudate from a destructive lesion in the anterior part of this nucleus. A radium tube (1 millicurie) was introduced thirty-three weeks before this brain was removed.

ness of its barking note. These signs all disappeared and the animal exhibited continuously a bright responsive behavior up to the time the autopsy was made, thirty-three weeks after the radium tube was introduced.

Discussion. A feature of interest in these experiments on dogs with lesions of the striato body relates to the motor control in walking. Attention has been called above to the conception that the corpus striatum is connected with such associated automatic activities as the synchronizing of the movements in walking of the hands and feet in man, or the fore and hind limbs in quadrupeds. This view receives some sup-

port from the fact that the motor patterns of these activities embody elements of both the aquatic and the terrestrial adaptation of living forms. Furthermore, the developmental history of the nervous system suggests that the paleostriatum is anatomically and physiologically connected with motor combinations of this type. A slightly different interpretation of this same idea is contained in the suggestion of Luciani and Tamburini (12), who assign to the caudate nucleus the function of control over those acts that are phylogenetically ancient.

In the experiments reported here especial care was exercised to detect any disturbance suggestive of a defect in the mechanism of associated control. About one-half of the animals evidenced a slight awkwardness when making slow walking movements. The first indication of this condition was observed about four days after the radium tubes were introduced, and usually it lasted from three to four weeks. Rapid movements, such as running or springing to catch food in the air, were performed without difficulty and apparently normal agility. In the light of these results, it appears that extensive striato destruction in dogs, of the type produced in these experiments, causes no significant defects in the associated control in the walking movements.

It has been shown that the maximum action of buried radium emanation tubes occurs within ten to fourteen days (13). The lesions produced in this way, therefore, are incisive in character and essentially nontraumatic. They are also uncomplicated in the sense that the etiologic factors do not act beyond the immediate region of the tube. In this regard they differ materially from striato involvement in man, in whom such things as vascular lesions and toxic and abiotrophic degeneration are looked upon as probable causative agents. By ablating portions of the striato body on both sides, as described above, we have elicited in all of the animals so treated characteristic tremors, confined mainly to the neck and shoulder region of the body. In addition to this there has been shown, both in animals with unilateral and in those with bilateral lesions, another sign of hypertonia in the form of a peculiar doubled-up attitude of the body. Unlike the tremor symptom, which gradually diminished and disappeared altogether within three to four weeks, the body attitude hypertonia was impressed on the behavior of two of these experimental animals for several weeks. It is of interest in this connection to note that clinico-pathological studies on human subjects have shown that extensive involvement of the entire striato body is accompanied by tremors and rigidity of the paralysis agitans type, tonic and clonic spasms and occasionally movements of a choreic

and athetoid nature. In our experiments some of these signs of striato motor disturbance have appeared. On the other hand, movements simulating the choreic and athetoid form probably find no counterpart in this form and have not been exhibited in any way.

Features of particular interest in the results of these experiments were: first, the comparatively small degree of alteration in the general behavior of these dogs following the destruction of large portions of the striatum, and, secondly, the capacity of compensating in a short time for any defects in motor control. In this connection it should be noted that no attempt was made to destroy the globus pallidus on account of its close anatomical relation to the internal capsule. It is not possible to determine to what extent the presence of the pallidum altered the nature and course of the neuro-physiological manifestations, since there is at hand no reliable experimental evidence with regard to the exact rôle in dogs of the neo- and the paleostriatum. Grunstein (14) has shown that lesions of the putamen are attended by some degeneration of the ansa lenticularis, which in a structural way would appear to connect this part with extrapyramidal control, and more recently Bielschowsky (15) has put forth the view that the putamen and caudate are intimately linked with and functionally control the globus pallidus. Considered in this light, the pallidum probably plays little part in the events that follow destruction of portions of the neostriatum.

Those signs of extrapyramidal release which we have observed in some of our animals we interpret as functionally demonstrating a form of motor control originating in the putamen and the caudate nuclei. But this type of control would appear to be very rudimentary, as evidenced by the fact that it was necessary to destroy quickly portions of both sides of the striato body in order to bring out the defect, and even then a transfer of function, either to undestroyed parts of the neostriatum or to other centers, readily took place.

The hypertrophic cirrhosis of the liver so commonly associated with progressive lenticular degeneration in man is a phenomenon of unusual interest. On anatomical and physiological grounds it seems very doubtful that the mechanism of association of these disturbances is one of involvement of the autonomic system, as suggested by Jelliffe (16). The present experiments furnish additional evidence, we believe, with regard to the incorrectness of his view, since ample time intervened after the destruction of the striatum for trophic changes of the liver to occur, but nothing suggestive of this condition appeared. The data at hand furnish no evidence concerning the alternate hypothesis of a toxic factor.

SUMMARY

1. A method is described for producing lesions in the corpus striatum by means of buried radium emanation tubes.

2. The lesions so produced were sharply localized (about 1 cm. in diameter), necrotic in character, and show accompanying phagocytic and edematous processes. The surrounding brain tissue was normal and some of the larger vascular trunks at the borders of the radiated fields were patent.

3. There was no tract degeneration in the spinal cord nor structural changes in the liver and other organs.

4. The destruction of large portions of the lenticular and caudate nuclei did not give rise to outstanding defects in the coördinated muscular activity of walking and jumping.

5. Extensive bilateral lesions, simultaneously produced, have been attended by tremors and signs of hypertonia exhibited in an unusual bodily attitude, with subsequent return to normal—or apparently normal—condition.

6. The treated animals were kept from two to thirty-three weeks. Some exhibited temporarily signs of dysphagia, disarthria, conjunctivitis, clumsiness in slow movements, tremor and hypertonia. All indications of the disturbance usually disappeared within four weeks.

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A STUDY OF THE RELATION OF AFFERENT IMPULSES TO THE ACTIVITY OF THE CENTRAL CARDIO-VASCULAR NERVOUS MECHANISM

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Received for publication March 5, 1923

Experiment shows that there is a clear relationship between impulses arising from experimental stimulation over specific afferent nerves and changes in heart rate and blood pressure. The question arises whether the maintenance of a certain degree of activity of the heart and blood vessels, as manifested by the maintenance of blood pressure, is dependent upon afferent impulses in any degree or wholly independent of them. In other words, is the maintenance of blood pressure a reflex or an automatic function?

There is no method previously employed for eliminating the afferent pathway from the heart to the central nervous system while leaving the efferent path intact. It is possible, however, to leave the afferent path intact and eliminate temporarily a part of the efferent pathway through the action of such drugs as atropine.

One may divide one or both of the vagi anatomically and note the changes in heart rate, or one may eliminate the efferent fibers of the vagi by the administration of atropine, leaving the afferent fibers intact, but neither method really gives any clear answer to the question whether the action of the vagus under normal conditions is reflex or automatic. The problem is further complicated by the presence of the accelerator fibers, and the effect of the elimination of the vagi may conceivably differ according to whether the accelerators have or have not previously been divided. There are, then, several possible conditions under which the effect of elimination of the cardiac nerves may be studied.

ANATOMICAL. 1. Division of both vagi, with the accelerators intact. This would include depressor fibers in some animals, but not in the rabbit.

2. Division of both vagi after previous removal of the stellate ganglia.

3. Atropine, vagi intact and accelerators intact.

4. Atropine, vagi intact but with previous removal of the stellate ganglia.

5. Section of accelerators, vagi intact.

6. Section of accelerators after division of both vagi.

7. Section of accelerators after administration of atropine.

The observance of all these conditions becomes necessary because of the possibility that the action of the accelerators may be related to afferent impulses over the vagus, since the existence of other true afferent cardiac nerves is doubtful. If afferent cardiac fibers do run in the accelerators, more conditions are introduced, all of which demand analysis. And to the various procedures given above, we must add division of the dorsal roots of the spinal nerves.

It may be remarked in passing that no complete experimental analysis of the problem has yet been made. And by the same token no clear answer can as yet be given to the question of the functional relation of afferent to efferent roots in the cardio-vascular system.

Just as it was necessary to inquire into the relations of afferent to efferent roots in the matter of the nervous control of the skeletal muscles in order to decide which of their responses were reflex and which automatic in nature, so we must go over the relation of afferent roots to efferent roots in the study of the cardio-vascular reactions. For purposes of simplicity and convenience in handling, we may consider separately the cardiac and vascular reactions, and then later try to get at the reciprocal reactions of the two parts of the same mechanism.

It has been shown that stimulation of the central end of the vagus, when one is divided and the other intact, slows the heart as a rule, although there is occasional acceleration of the heart instead. It has been shown also that the vagus is the only afferent nerve stimulation of which will consistently slow the heart. There are both afferent and efferent fibers in the vagus and it has been impossible up to the present to separate these anatomically. The determination of the relation of one set of fibers to the activity of the other must be arrived at by some other more round-about and possibly less certain method.

The usual effect of section of both vagi is an increase in the rate of the heart and a rise of blood pressure. This simple experiment does not, however, throw much light on the question of whether the normal action of the vagus in keeping the heart rate down is reflex or automatic in character. In order to determine this point, further experimentation is necessary. It becomes necessary, therefore, to observe the conduct of the heart when the vagus is severed under a number of different conditions, which have been stated above.

STATEMENT OF PROBLEM. Sherrington (1) has considered a funda mental characteristic of the responses of the central nervous system under the caption of the relation of afferent to efferent roots. The greater volume of work on this relationship has been devoted to the conditions prevailing in the somatic rather than in the visceral sensory and motor systems. While the responses of the somatic system are necessary for locomotion and other activities, and certain reactions of the organism to some conditions or changes of conditions in the external environment, the responses of the visceral system are necessary for the maintenance of the constancy of the internal conditions of the higher organism, such as systemic blood pressure, as well as for the adjustment of internal conditions in relation to the changes of the external conditions. The question arises whether the responses of the visceral system involve this relationship of afferent to efferent roots to the same degree as the somatic systems.

Sherrington considers this question to some extent in his chapter (loc. cit.) but the degree to which this relationship holds in such a nervous mechanism as that for the control of respiration or blood pressure is not answered. Nor does one find this answer in any of the papers so far published. It is evident that for the proper understanding of the function and manner of action of any of these visceral systems, the ascertainment of the degree to which the relation of afferent to efferent roots holds is a necessary preliminary.

It may be pointed out here that just as the changes of internal conditions, practically independent of afferent impulses, may affect the respiratory rate, so it is possible that changes of internal conditions which are not primarily related to afferent nervous impulses may affect cardio-vascular activity. The relation of afferent to efferent roots may then be masked or modified by other internal conditions in the case of both of these systems, and the answer to our question may at first involve the indirect quantitative estimation of a number of factors in an equation rather than the direct determination of one of them.

HISTORICAL. Beginning with the discovery by the Weber brothers of inhibition of the heart by applying one electrode to the spinal cord at the level of the 4th or 6th vertebra and the other to the nostril of a frog, and the later discovery of an inhibitory center by the same workers and others, the question as to whether this center and the vagi could be affected reflexly or not has been very much under discussion. Even after the vagus center had been definitely and minutely located in the medulla, very little progress had been made in regard to this question.

As a result, the mechanism of the nervous control of the entire cardio-vascular system was more or less in the dark.

That the vagus center may be reflexly stimulated by stimulation of various afferent nerves, and that vagal tone may be altered by the blood itself has been shown. That psychical and emotional states could change vagal tone was also shown by many workers. By stimulation of the cerebrum itself marked changes in the pulse rate may occur. But all this work did not show whether the tone maintained by the vagus center is kept up reflexly by constant instreamings of afferent impulses, or whether the center had the power of automatism. These experiments do not, for instance, indicate what effects changes in the internal environment of the organism may produce on the center.

That changes in the internal environment of an organism may have profound effects on pulse rate and blood pressure of an animal is very clearly shown by the changes in heart rate which follow deep inspiration and deep expiration. At the end of deep inspiration a marked increase in heart rate is observed as well as a marked increase in arterial tension; while a marked decrease in heart rate and a fall of arterial tension is observed at the close of deep expiration. The effect of any increased muscular work will change the internal environment to a certain degree, and this change may be accompanied by very great changes in the cardio-vascular system. This phase of the question has been largely overlooked by previous workers as well as by those who, until lately, have been working on the nervous mechanism of respiration. That vagal tone is maintained reflexly has been shown by some workers, such as Soltman, but the evidence presented by them is neither very clear nor very conclusive. In a similar manner evidence has been presented against this view which also lacks in conclusiveness. There is the work of Hill who makes the supposition that the terminations of the afferent nerves on the inner wall of the heart may be stimulated by intracardiac pressure and thus control the centers of circulation. That this view is perfectly tenable can be shown by the experiments of Johanssen (2) and many others who, after section of all the cardiac nerves, observed a rise of arterial tension and an increased heart rate on compression of the abdominal aorta. In the absence of the afferent pathways no stimulation arising from pressure in the heart can be effective reflexly. If however all the cardiac nerves are intact, Marey (3) noted that the compression of the abdominal aorta is followed by a decreased heart rate. Whether the central mechanism of the vagi is in this case affected by afferent impulses or by the increased intracranial pressure cannot be

determined. The heart, through the depressor nerves, has afferent connections with the central mechanism and by this means it is perfectly possible for it to control reflexly its own rate.

Sherrington (4) has sectioned all the dorsal roots of an animal in which he was recording blood pressure without getting any appreciable change in pressure. This fact seems to demonstrate that the vagus center is not dependent on afferent impulses coming in over the dorsal root fibers since, when all the afferent impulses, except those over the vagi, had been prevented from reaching it, there was little change in blood pressure.

Stewart, Guthrie, Burns and Pike (5) have shown that after a total loss of function of the cerebral centers resulting from the temporary ligation of the head arteries there is a gradual return of function following the restoration of the circulation and that this return occurs in a fairly definite order. In a second publication, Stewart and Pike (6) reported a more detailed study of the time relations of the resuscitation processes in the most important of the bulbar centers, namely, the respiratory, vasomotor and cardio-inhibitor. Since by the production of complete cerebral anemia all the reflexes of the anemic region are temporarily abolished, advantage was taken of the opportunity afforded for the study of the relation between the return of such functions as respiration, or cardiac inhibition, and the opening up of the reflex pathways to or through their centers. In regard to the respiratory mechanism these authors found it was, of the three, the most automatic and the least dependent upon reflex stimulation for the origination of its discharge. The cardio-inhibitory center was the least automatic and is the most dependent upon such impulses while the vasomotor mechanism occupied an intermediary position between the other two mechanisms. The cardio-inhibitory center regains its power of being affected by reflex stimuli sooner than it does its tone.

Stewart (7) has also shown that in dogs the rate of the heart when isolated from its extrinsic nerves by cerebral anemia is relatively constant when the external conditions (temperature and pressure of the blood) are kept constant.

The rôle played by the accelerators is of course extremely important in a consideration of the cardio-vascular system. That they are purely efferent nerves has been generally believed, but whether they are dependent on afferent impulses or whether they work automatically has not been definitely determined. Tschirjew, Stricker and Wagner have witnessed, after section of the vagi, a decreased pulse rate on

excision of the inferior cervical and the first thoracic ganglia. It is also claimed that after section of the accelerators, inhibition by excitation of the vagus can be induced with great ease. Timofeew has kept animals for some time after section of the accelerators and noticed in them a gradual decrease in pulse rate. This fact evidently points to a tonic action maintained by the accelerators but it is held in check by the greater tonicity of the vagus center. It affords evidence also that the accelerators hold the vagus mechanism in check.

Hunt and others have suggested that the tone maintained by the accelerator nerves is on blood pressure, since extirpation of the accelerators or the stellate ganglia is followed by a fall in blood pressure, in some cases amounting to 50 per cent.

The question as to whether reflex cardiac acceleration may occur independent of the cardio-inhibitory center is one which has been fairly well investigated but on which there still exist many contradictory statements. There are two possible conditions under which reflex cardiac acceleration may occur: *1*, through a depression of the vagus action; or *2*, through a stimulation of the accelerator center. A great many investigators seem to favor the former view. Roy and Adami (8) conclude that the acceleration of the mammalian heart may occur only through the vagus center. Hunt confirms this view.

In opposition to this view are those who maintain that a reflex cardiac acceleration may occur independent of the cardio-inhibitory center. Among these are Francois-Franck (9) who states that although the vagus center predominates over accelerator action under normal circumstances yet the reverse may be true under experimental conditions. Von Cyon (10) and Bayliss (11) also accept the view that reflex cardiac acceleration may take place through a stimulation of the accelerator center. The latter author has stimulated the depressor after section of both vagi and obtained an accelerated heart. Hering (12) observed in rabbits in which the last cervical and first thoracic sympathetic ganglia had been extirpated, that cardiac acceleration following muscular exercise was very much more reduced after than before the operation. That the second view is more tenable has also been confirmed by Hooker (13).

Gasser and Meek (14) claim that the acceleration of the heart at the beginning of voluntary exercise in the normal animal is chiefly due to the decrease in the tone of the cardio-inhibitory center. Their work leads them to believe that the accelerators are a factor of safety and that, in exercise, their action is superimposed on that of the vagi only in times of great need. Aside from this these authors claim that their chief function is to maintain the level of the resting pulse.

EXPERIMENTAL PROCEDURE. All the experiments were done on cats. The laboratory procedure was ether anesthesia and tracheotomy. Blood pressure was recorded by means of a mercury manometer connected to the left carotid artery. Records were made on a Hürthle kymograph. The time was recorded in seconds by a Jacquet chronograph. A signal magnet served as the base line for blood pressure. At all times a sufficient amount of ether was given to abolish all sense of pain in the animals experimented on. The depth of anesthesia was judged by the presence or absence of the corneal reflex. The heart rate was computed in beats per minute. In every case a control tracing of blood pressure and heart rate was taken before any other procedure was attempted.

Atropine was administered either directly intravenously or indirectly by stomach tube. In the former case the femoral vein was dissected out and a cannula was inserted into it. Atropine was injected by means of a hypodermic syringe, the needle of which was plunged into a rubber tube attached to the cannula. Backflow was prevented by a clamp on the free end of the rubber tube. In the latter case, a rubber tube was inserted into the esophagus until it reached the stomach. The drug was injected by plunging a hypodermic needle directly into the rubber tube.

The solution of atropine was made up by weighing out a definite amount of atropine sulphate and dissolving it in a definite amount of one-seventh molecular solution of sodium chloride. In this way it was known how many milligrams each cubic centimeter of solution contained, and the dose was injected accordingly. The solution kept very well in a stoppered bottle at room temperature for several days. The dose injected was about 2 mgm. contained in 1 cc. of saline solution. This amount was, however, not strictly adhered to, since the animals varied from half-grown to full-grown cats.

I. DIVISION OF BOTH VAGI WITH THE ACCELERATORS INTACT. In animals such as the rabbit in which the depressor fibers of the vagus of most individuals run in a separate strand, the effect of the division of the vagi may be observed independently of the effect of the section of the depressor fibers. In the cat and the dog, this separation of the two kinds of fibers cannot be made so easily. As has been indicated, section of the vagi may be done with or without the concomitant division of the depressor according as the rabbit or some other mammal is used for the experiment. In cats, which were used throughout these experiments, bilateral vagotomy is followed usually by an increase of heart rate and blood pressure. The effect varies somewhat with the general systemic blood pressure at the time the section is made and with the general

depth of anesthesia. If for any reason the heart rate is abnormally high, there is no increase on division of the vagi.

In some of the cats used, separate depressor fibers were found, but the number was so small that even in those animals in which the separate depressor fibers were found the section included these fibers. It might be added here that very often a great deal more trouble was encountered in animals with separate depressor nerves than in those in which these fibers run in the vagus. Not only was this my experience but also that of Doctor Coombs who was doing experiments in this laboratory at the time this work was in progress.

Both vagi, after being carefully dissected out, were exposed for a distance of about 2 cm. and then loose ligatures were passed around them. The section of both nerves was made practically simultaneously. The results of double vagotomy are found in the table below, which gives an average of about fifteen such experiments.

TABLE 1

	RATE	PRESSURE
Before division of both vagi	212	136
After division of both vagi	229	151
Percentage change	8	11

In practically every case studied, bilateral vagotomy was followed immediately by a rise of systemic pressure and an increased heart rate. In some instances the rise in pressure was preceded by a slight fall which was probably due to a stimulation of the efferent fibers of the nerves during the process of lifting them or by the very act of cutting them. This initial fall was very slight, however, and was immediately followed by the usual rise. It will be observed on consulting the table that there is a change both in heart rate and blood pressure.

In some animals section of both vagi was frequently followed by a great impairment of respiration. The animals had great difficulty in respiration, and very often artificial respiration had to be given for several minutes. In some animals the blood pressure fluctuated greatly for some time after bilateral vagotomy, but after a short time the usual rise in pressure and acceleration of the heart was obtained, and this was maintained fairly uniformly until the end of the experiment.

II. DIVISION OF BOTH VAGI AFTER PREVIOUS REMOVAL OF THE STELLATE GANGLIA. The method used in the dissection for the stellate

ganglia was suggested by Professor Pike. It is safer than any of the other methods tried since the chances of puncturing the pleura and producing a pneumothorax are much lessened by this method.

The pectoralis muscles were dissected away. The initial incision was made in the midline of the sternum, thus avoiding most of the large blood vessels. After this dissection, the ribs and the intercostal muscles were exposed. The next group of muscle to be dissected away was the chief muscles of the neck which included the cleidomastoid, sternohyoid, sternomastoideus, sternothyroideus and the scalenus. The trachea and the esophagus were thus exposed. With a pair of pointed forceps around which a small piece of cotton was wound, the highway of the esophagus was followed until a point where merely moving the esophagus slightly aside revealed the inside of the thorax. The stellate ganglia lie between the first and second ribs, and merely pushing the muscles aside reveals them. With a fine pair of pointed forceps the ganglion was raised and then removed with a fine pair of scissors.

Removal of both stellate ganglia is immediately followed by a considerable fall of blood pressure, at times more than 50 per cent. The heart rate slows and neither pressure nor rate increases. If both vagi are sectioned following the removal of the stellates there is a gradual rise of blood pressure which, however, never reaches the pressure which was maintained before the stellate ganglia were removed. There is also a striking increase in heart rate. It would seem as if section of the vagi allows the heart to escape from the tone which these nerves maintain over it. However, the rate which was maintained at the beginning of the experiment is never regained, as reference to table 2 will show.

It will be seen, therefore, that the removal of the stellate ganglia is followed by an immediate large fall in pressure, to a level which is maintained throughout the experiment provided no other lesions are made. The heart rate also decreases and this decrease is constant under the same conditions. But if bilateral vagotomy is now done, the pressure goes up somewhat but never reaches the normal level. The same is true also of the heart rate. In other words, it would seem as if blood pressure and heart rate are maintained by the stellate ganglia and the accelerator nerves while the vagi are concerned with keeping up a slight tonic action on blood pressure, but that their main function is to keep the rate of the heart down so that it is not overworked.

Hunt (15) has shown that the maintenance of a certain degree of blood pressure seems in some way to be connected with the stellate ganglia as is shown by what happens when they are removed, for then

both the rate and the pressure fall. Wickwire (16) has shown that the removal of the accelerators alone results in a moderate decrease in heart rate and a great fall in pressure.

Reference to the following table will bring out the points mentioned above more clearly.

III. Atropine: Vagi and accelerators intact. Atropine is an alkaloid which paralyzes the terminations of a number of nerves, more especially of those of autonomic, as distinguished from sympathetic, origin that supply the involuntary muscles, secretory glands and the heart. By virtue of this property, it paralyzes the inhibitory terminations of the vagus in the heart and stimulation of this nerve therefore causes no change or at least, no decrease of the pulse after its administration (17). Small quantities of atropine have no further action on the heart than the paralysis of the inhibitory nerve endings. The terminations of the accelerator nerve endings are not affected. The heart

TABLE 2

	RATE	PRESSURE
Before removal of stellate ganglia	173	147
After removal of stellate ganglia	149	92
After division of both vagi	160	105
Percentage change when the stellate ganglia are removed first	8	29

therefore after the administration of this drug is in a position as if the efferent fibers of the vagi had been cut in the neck. This results in a marked quickening of the heart.

In these experiments atropine was at first injected intravenously. In each case the heart was markedly accelerated. At the beginning there was always a decreased pressure which is probably due to the action of the drug on the heart musculature itself. In later experiments atropine was given by stomach tube so that its absorption into the blood stream was much slower than when given directly intravenously. In such cases besides getting the acceleration of the heart, a slight rise of blood pressure was also obtained. In other words, administration of atropine in small quantities into the blood stream has the same effect as bilateral vagotomy except that the afferent pathway of the vagi is open. Table 3 will show this fact more clearly.

Schiff (18) about fifty years ago maintained that stimulation of the peripheral end of the vagus nerve after atropine had been administered,

always resulted in an increased heart rate. In other words, he showed that the vagus contains accelerator fibers and that after atropine was injected the inhibitory fibers were paralyzed while the accelerator fibers were not acted upon, so that stimulation of the peripheral end of the cut vagus resulted in a stimulation of the accelerator fibers with the result that the heart rate was accelerated. Arloing (19) also maintained that there were accelerator fibers in the mammalian vagus. I repeated this experiment and obtained the same results. In every case, practically, stimulation of the peripheral end of the cut vagus after atropine had been administered gave an increased heart rate.

Another point of interest which was brought out in these experiments is the fact that the accelerators are receiving afferent impulses from the vagi and that therefore there is a definite relationship between afferent

TABLE 3

	RATE	PRESSURE
When injected intravenously:		
Before injection of atropine	183	145
After injection of atropine	195	122–106–97–92–83
When injected by stomach tube:		
Before injection of atropine	210	127
After injection of atropine	216	132
Percentage change	3	4

to efferent roots in the maintenance of blood pressure. The following series of experiments will tend to bring this point out.

Atropine was administered either directly intravenously or by the stomach tube, and sufficient time was given for its absorption and action.

The vagus contains both afferent and efferent fibers but only the latter are affected, leaving the afferent pathway intact. After the lapse of a short time either the right or the left vagus was sectioned, and its central end ligated. A medium tetanizing current was then used to stimulate this central part of the nerve. If the stellate ganglia and the accelerator nerves were intact a rise of pressure was always obtained. If however the accelerators had been removed, this rise in pressure was never obtained, but a fall was the rule. In other words afferent impulses from the vagus nerves reflexly affect the central accelerator mechanism, so that this is in a measure dependent for its activity on the afferent impulses coming over the vagi.

IV. ATROPINE: VAGI INTACT BUT WITH PRIOR EXCISION OF THE STELLATE GANGLIA. In this series of experiments excision of the stellate ganglia was immediately followed by a great fall of pressure and a decreased heart rate. After the administration of atropine, either intravenously or by the stomach tube, the blood pressure rose slightly while the heart rate also increased. Administration of atropine after the removal of both stellate ganglia gives results somewhat similar to those following bilateral vagotomy after excision of the stellate ganglia. This series of experiments again illustrates the fact that the maintenance of a certain degree of blood pressure is dependent upon the accelerators, while the vagi maintain a slight tonic action on blood pressure after the removal of the accelerators or the stellate ganglia. Likewise a tonic action is maintained by the vagi on heart rate, for after their section or paralysis by atropine the heart is immediately accelerated, although the rate maintained at the beginning of the experiment is never reached, if

TABLE 4

	RATE	PRESSURE
Before excision of the stellate ganglia	202	152
After excision of the stellate ganglia	184	82
After the injection of atropine	190	115
Percentage change	6	24

the vagi are sectioned or paralyzed by atropine subsequent to the excision of both stellates.

Stimulation of the central end of the right or left vagus with the other one intact, and with atropine administered, but with the stellates removed, results in a fall of pressure, whereas with the stellates intact the usual rise of pressure is obtained. This again illustrates the fact that the central accelerator mechanism is in a measure dependent for its activity on the afferent impulses coming over the vagi.

V. SECTION OF ACCELERATORS, VAGI INTACT. Section of the accelerator branches from the stellate ganglia results in a fall of pressure. The heart rate however does not seem to be reduced as much in this case as when the stellate ganglia are completely removed, in which case there is a great fall in pressure and heart rate. With the accelerator branches alone sectioned, the pressure subsequently goes up slightly and with it the heart rate is somewhat accelerated. This is possibly, even probably, due to accelerator impulses reaching the heart through the cervical ganglia (20), (21).

VI. Section of accelerators after division of both vagi. Section of both vagi is followed by the usual train of events, namely, an accelerated heart and a rise of blood pressure. This increased heart rate and blood pressure is maintained if no other experimental lesions are made, but if the accelerators are sectioned subsequent to bilateral vagotomy there is a fall of pressure and a decreased heart rate. The fall of pressure due to section of the accelerators is not as great in this case as in the case where the stellate ganglia are first removed and double vagotomy done subsequently, but the change in heart rate in the first case is greater than in the second case. It would thus appear that the ultimate heart rate and the ultimate blood pressure depend considerably on whether the accelerators or the vagi are sectioned first.

TABLE 5

	RATE	PRESSURE
Before accelerators are sectioned	158	147
After accelerators are sectioned	156	95
Percentage change	2	35

TABLE 6

	RATE	PRESSURE
Before the division of both vagi	202	138
After the division of both vagi	218	152
After section of the accelerators	180	105
Percentage change	11	24

With the ultimate heart rate the opposite seems to be true, namely, there is a greater final fall in heart rate when the vagi are sectioned first and this procedure followed by section of the accelerators than when the accelerators are sectioned first and this procedure followed by double vagotomy.

VII. Section of the accelerators after administration of atropine. In practically all respects this series of experiments agrees with the results that would be obtained if the vagi had been sectioned and this procedure followed by the removal of the accelerators. The percentage changes, however, were not quite as large. Upon the administration of atropine the blood pressure rose and the heart rate was increased. Now if the accelerators are removed, blood pressure falls

while the heart rate decreases. It would seem that if the vagi are first paralyzed by atropine or sectioned, the subsequent removal of the accelerators is followed by a smaller fall of blood pressure and a slightly greater decrease in heart rate. Immediately upon the removal of the accelerators, the pressure falls; in time the pressure gradually rises, but the initial pressure and the heart rate are never reached again.

It would thus appear that when all the extrinsic cardiac nerves are cut, either the heart rate falls somewhat and blood pressure considerably or there may be a greater decrease in heart rate and only a moderate fall of blood pressure. The final result seems to depend on whether the vagi or the accelerators are removed first. Table 7 will bring this point out clearly.

VIII. Section of the vagi subsequent to ligation of the adrenals. This series of experiments was undertaken for the purpose of seeing whether adrenalin played any part in the maintenance of blood

TABLE 7

	RATE	PRESSURE
Before the administration of atropine	210	127
After the administration of atropine	216	132
After the accelerators are removed	194	105
Percentage change	10	20

pressure under the conditions given. In every case that has yet been investigated, the action of adrenalin on muscle is identical with that produced by stimulation of the sympathetic nerve supply to that tissue (22). Since the accelerators belong to the sympathetic nervous system, it was thought that there might possibly be some relationship between their influence over blood pressure and the amount of adrenalin secreted. The usual procedure in dissecting out the adrenals was to make an incision in the mid dorso-ventral line near the region of the kidney, great care being taken not to injure the diaphragm. The fat and fascia around the adrenals were carefully dissected away leaving the gland free all around. The adrenal lumbar vein which carries away the secretion of the gland was tightly ligated and then to make doubly sure a ligature was passed around the entire gland thus shutting it off completely from the vein. In no case was there a sufficiently large hemorrhage to cause any change in heart rate or blood pressure.

Immediately after ligation of the adrenals there was a small fall in pressure in every one of the series of animals studied.

The next procedure was to section both vagi. In these experiments it was desired to see whether or not the accelerators cut off from a supply of adrenalin were able to cause the usual increased blood pressure and the usual increased heart rate, which is obtained after double vagotomy under normal conditions. Immediately following double vagotomy an increased blood pressure was obtained which lasted for a few seconds only, and then the pressure began to fall. The heart rate was however slightly increased both after ligating the adrenals and after double vagotomy.

IX. PARTIAL CEREBRAL ANEMIA FOLLOWED BY THE REMOVAL OF THE STELLATE GANGLIA AND SUBSEQUENT SECTION OF THE VAGI. When, for any reason, the volume of blood circulating through the brain in unit time is greatly reduced, there is a cardio-vascular reaction in a direction tending to restore the normal volume conditions in the brain. Blood pressure rises markedly, and one often finds a departure from the usual

TABLE 8

	RATE	PRESSURE
Before ligation of the adrenals	210	154
After ligation of the adrenals	226	144
Immediately after double vagotomy		160
A few seconds after double vagotomy	231	135

relations expressed in Marey's law, of a slower pulse rate with a high blood pressure. The vasoconstrictor part of the response has been studied by Winkin (26) and it seems desirable to get some further evidence upon the more strictly cardiac changes under conditions of reduced blood supply to the brain, and the rôle payed by the cardiac nerves in the response of the organism to such a change of internal conditions.

A state of partial cerebral anemia was induced by the occlusion of the right carotid artery, and the right and left vertebral arteries, but allowing a small amount of collateral circulation. Ligation of these cerebral arteries and the production of a state of partial cerebral anemia is followed in some animals by a fall of arterial pressure instead of the usual rise of pressure. This response was observed in a number of animals in this series of experiments. This condition can be remedied by releasing one of the vertebrals, usually the left. In this series of experiments the stellate ganglia were removed before the production of partial

cerebral anemia. The usual fall of blood pressure and heart rate was observed following the removal of the accelerator influences on the heart. Now when the cerebral arteries were occluded, with the exception of the collateral circulation mentioned above, the pressure rose while the heart rate slightly decreased. On releasing the carotid, the pressure fell to slightly below normal. The second occlusion was also followed by an increased blood pressure, but in this case the pressure did not rise as high as in the first occlusion, while the heart rate slightly diminished. The third occlusion and every occlusion following was accompanied by a gradual decrease in blood pressure and heart rate.

Section of the vagi during the period of partial cerebral anemia is followed by a great increase in blood pressure and heart rate as compared with blood pressure and heart rate when the vagi were still intact. Especially was there an increase in heart rate as the table below will show. Occlusion of the cerebral arteries is now followed by a pressure and rate much greater than in the case when the vagi were intact. The second and subsequent occlusions result in a rise of pressure which is much greater than in the previous case when the vagi were intact. In the case of the heart there is also an increase over the rate present when the vagi were intact, but the rate is not as high as the rate observed at the beginning of the experiment.

TABLE 9

	RATE	PRESSURE
Normal	225	130
Stellates removed	169	94
1st occlusion	183	155
2nd occlusion	173	141
3rd occlusion	160	132
Vagi sectioned	203	180
Normal circulation restored	193	133
1st occlusion	198	172
2nd occlusion	198	170
End of the experiment	188	115

A higher rate of the heart at the beginning of the experiment, than is ever attained after section of the accelerators and the vagi, indicates a tonic action of the accelerators. This is not consistent with the results of Gasser and Meek who deny the tonicity of the accelerator nerves.

X. Partial cerebral anemia followed by section of the vagi, stellate ganglia intact. A condition of partial cerebral anemia was induced as described above, with all the cardiac nerves intact. The usual rise of pressure and the slightly decreased heart rate are observed. The primary decrease in heart rate is usually followed later on by an increased heart rate if the condition of partial cerebral anemia is allowed to remain without any further lesion. This later acceleration might be explained by the following. Schiff showed that the accelerator fibers of the vagus resist degenerative changes longer than the inhibitory fibers. Arloing (19), who repeated Schiff's work, says there are four kinds of fibers in the vagus, one kind being the accelerator fibers, and confirms Schiff's work as to their greater resistance to degeneration. Other experiments have shown that the accelerator fibers retain their vitality longer after death of the animal than the inhibitory fibers. It is conceivable therefore that the accelerators are more resistant to anemia than the inhibitory fibers. Stewart, Guthrie, Burns and Pike (5) and Winkin (26) have also shown that there is a vasoconstriction in the splanchnic area during cerebral anemia. The rise in pressure and the accelerated heart may perhaps be attributed to the action of the vasoconstrictor mechanism and in part to the action of the accelerator fibers in the vagus.

The results of sectioning the vagi during a condition of partial anemia depend on whether the accelerators are present or not. If the accelerators are present, the pressure rises very high on partial occlusion of the cerebral arteries. On releasing the head arteries, the pressure never falls below the control pressure maintained by the animal; whereas if the accelerators have been excised, the pressure does fall below the control level. If the vagi are sectioned when the accelerators are not present, and then the cerebral arteries are occluded, the pressure does not rise very high and following the release the pressure falls below the control level. The accelerator mechanism seems to stand in the way of a falling blood pressure and saves the cells from a deficiency in the amount of blood circulating to them.

A contrast of the deportment of the heart when the vagi are sectioned during a state of partial cerebral anemia with the accelerator mechanism intact and with the accelerator mechanism removed is shown by the results of the following two experiments.

	BLOOD PRESSURE
Normal	122
Cerebral arteries partially occluded after section of the vagi with the accelerators intact	210
Normal circulation restored	125
Normal	130
Cerebral arteries partially occluded after section of the vagi and removal of the accelerators	140
Normal circulation restored	105

Section of the vagi during a state of partial cerebral anemia with the accelerators intact is followed by a great increase both in blood pressure and the heart rate. On releasing the cartoid the pressure and heart rate fall to a little above that maintained at the beginning of the experiment.

TABLE 10

	RATE	PRESSURE
Normal	197	128
After occlusion	191	197
Vagi sectioned	214	207
1st occlusion	217	207
2nd occlusion	212	206
3rd occlusion	212	207
4th occlusion	210	206
Normal circulation restored	200	135

XI. PARTIAL CEREBRAL ANEMIA FOLLOWED BY SECTION OF THE VAGI AND SUBSEQUENT REMOVAL OF THE STELLATE GANGLIA. In this case, section of the vagi is followed by the increased pressure and heart rate described in the preceding table. Now if the stellates are removed the pressure falls very much below normal, while the heart rate also decreases. If a state of partial cerebral anemia is now induced by occluding the cerebral arteries, leaving a slight collateral circulation by way of the left vertebral artery, the pressure increases, while the heart rate remains about the same. The second and subsequent occlusions raise the pressure to about the same degree as the first occlusion, and the heart rate remains constant. It would seem as if the removal of the stellate ganglia had removed the mechanism by means of which a high blood pressure can be obtained, when it is to the best interest of the organism to have a higher pressure than is present. The absence of

this mechanism may then be injurious to the best interest of the organism when a high blood pressure and an accelerated heart rate are necessary.

XII. Section of the vagi during complete cerebral anemia. In all the experiments previous to this, the vagi were sectioned either when the entire circulation was intact or during a state of partial cerebral anemia when a certain amount of collateral circulation to the head was still open. In each case a rise of blood pressure and an increased heart was observed. Whether this effect was due to central or peripheral forces could not be determined under these conditions, and so the same experiment was repeated with complete cerebral anemia. The cerebral anemia was considered complete when all voluntary and involuntary activities had ceased. When the hind limbs became quiet, and the

TABLE II

	RATE	PRESSURE
Normal	184	130
After occlusion	168	154
Vagi sectioned	206	197
1st occlusion	206	196
2nd occlusion	202	195
3rd occlusion	204	197
Stellates removed	184	94
1st occlusion	184	122
2nd occlusion	184	122
3rd occlusion	186	122

blood pressure began to fall, complete cerebral anemia was present. If the vagi are sectioned during complete cerebral anemia, there is absolutely no change in blood pressure or heart rate as compared to that present before the section was made. If a state of cerebral anemia however, is not present, as in the case when the circulation to the head is not interfered with at all, section of the vagi is followed by a rise of blood pressure and an increased heart rate.

This experiment then shows that the rise in blood pressure and increased heart rate, which are observed on sectioning the vagi, are a central effect and are not due to any peripheral activity for when the entire brain is made functionless by depriving it of its normal blood supply, the reactions that normally occur when the blood supply to it is intact, such as an increased blood pressure and heart rate, are abolished.

DISCUSSION OF RESULTS. At the beginning of this paper it was stated that the object of these experiments was to determine whether there was any relation of afferent to efferent roots in the maintenance of blood pressure or whether this function was entirely automatic. The central respiratory nervous mechanism and the central cardio-vascular mechanism function so nearly alike in this respect that it would be well to state here the views of Pike and Coombs (23) in regard to the nervous control of respiration. These authors in a brief summary of the organization of the nervous mechanism of respiration conclude as follows. "While it is undoubtedly true that the respiratory center may act automatically under conditions which preclude the effect of afferent impulses, it is our opinion that its normal activity is not wholly automatic but partly reflex." In various publications the same authors have shown that afferent impulses reach the respiratory center in higher mammals from the lungs and the respiratory epithelium generally, parts of the alimentary epithelium, the pleura, the muscles of the thoracic

TABLE 12

	RATE	PRESSURE
Normal	168	150
Complete cerebral anemia	160	200
Vagi sectioned	160	200

walls, the diaphragm and the abdominal muscles. If the vagi are sectioned the rate of respiration is slowed for the reason that afferent impulses coming over the vagi, which are normally summed with the carbon dioxide in the blood to produce an excitation of the cells in the medulla, are no longer present, and the excitation of the central cells is now dependent in large part upon the carbon dioxide only. If now the dorsal roots of the spinal nerves are sectioned, there is a further fall in the respiratory rate, due to a greater loss of afferent impulses to the central cells in the medulla. The cells in the medulla now become wholly dependent upon the carbon dioxide of the blood.

Briefly stated, then, the respiratory center normally receives afferent impulses from certain sensory fields. If these afferent impulses are prevented from reaching the central cells in the medulla by sectioning the vagi and the dorsal roots of the spinal nerves, respiration does not cease but continues in a modified form. The same statement can be made in regard to the central cardio-vascular mechanism. If all the

cardiac nerves are sectioned, the blood pressure does not drop to zero, but it is maintained at a certain level which is not the same as in the same animal with the cardiac nerves intact.

The nervous centers concerned with the regulation of the cardio-vascular mechanism are situated in the medulla and have a wide relation with afferent nerve fibers from all parts of the body including the heart itself. To the heart go efferent fibers which carry impulses from the central mechanism which increases or decreases its rate, and to the blood vessels go fibers which may change their caliber and thus change blood pressure. Sherrington and Wickwire have shown that section of all the dorsal spinal roots has no effect on blood pressure, and so we may concern ourselves with the changes brought about by sectioning the cardiac nerves themselves.

The problem of the relation of afferent impulses to the activity of the central cardio-vascular nervous mechanism may be divided into two parts. *1*, The determination of whether or not blood pressure may persist after section of all the afferent nerves; and *2*, the character of the response to changes at the periphery. There is little doubt that the respiratory mechanism in the medulla may function in the absence of all afferent nervous impulses. But it is equally clear that under these conditions no modifications of the respiration can be brought about by peripheral changes which cannot affect the central mechanism directly or indirectly through the blood. It is possible, also, that blood pressure may be maintained in the absence of all afferent nervous impulses, but the absence in such a steady uniform condition of any characteristic modification such as changes in the rate of respiration, would mask any noticeable manifestations of the effect of elimination of the afferent nervous impulses. One would expect, however, a failure of the usual responses to peripheral changes.

That a definite relation between afferent and efferent roots exists in the maintenance of blood pressure has been shown in an earlier part of this paper. If atropine is injected, thus paralyzing the efferent fibers of the vagi, and then the central end of the cut vagus is stimulated, a definite rise of blood pressure is obtained if the stellate ganglia and the accelerator nerves are intact; but a fall of pressure is the rule if the latter are removed. In other words, afferent impulses over the vagi reflexly affect the central accelerator mechanism.

That blood pressure does persist after section of all the afferent nerves has already been shown. In this respect the cardio-vascular system may be compared to the respiratory system, since respiration will go on

even after all the afferent nerves are sectioned, but in each case we get a modified type of respiratory or cardio-vascular response. This indicates that a certain amount of automatism is present in both these systems, but does not preclude the presence of the relation of afferent to efferent roots also.

As to the character of the responses at the periphery after the elimination of the afferent cardiac nerves, experiments indicate that there is a failure of the usual responses at the periphery. If the cerebral arteries are partially occluded when the vagi are intact, we get a rise of pressure not very much above the normal pressure while the heart rate decreases somewhat. If now the vagi are sectioned and afferent impulses from the heart can no longer reach the central cells in the medulla, a state of partial cerebral anemia results in a great rise of blood pressure and an increased heart rate. Wickwire (16) in a study of the reciprocal reactions of the cardio-vascular system has shown that when the vagi are intact compression of the abdominal aorta is followed by a compensatory response of heart rate to changing blood pressure, the magnitude of the response varying with the depth of anesthesia. The carotid blood pressure increases while the heart rate decreases. If the same experiment is carried out under similar conditions but with bilateral vagotomy, the compensatory response of a lower heart rate to increased blood pressure remains, but the degree of compensation is much less than before division of these nerves. Yates (24) has also found that if the medulla is intact, stimulation of the brachial plexus results in a rise of blood pressure. If the medulla is eliminated by producing a state of cerebral anemia the stimulation of the brachial plexus produces no rise of blood pressure. It seems safe to conclude that the response at the periphery seems to depend on whether afferent impulses can reach the central cardio-vascular mechanism. If these afferent impulses are prevented from reaching the medulla by sectioning the afferent nerves or by the production of a state of cerebral anemia the usual response at the periphery changes or fails entirely.

It will be observed on consulting table 1 that if the vagi are sectioned there is an appreciable change in both rate and blood pressure. If afferent impulses, however, can reach the central mechanism as in the case where atropine is injected, the change in rate and pressure is not so large as is shown by table 3. It would seem that afferent impulses from the heart over the vagi are necessary to keep the rate and pressure down to a normal level so that the cardio-vascular system is prevented from maintaining too high a rate and pressure. In the case where the

vagi are sectioned after both stellates are removed we also notice a greater fall in both rate and pressure than when, instead of sectioning the vagi, atropine is injected. In the latter case the afferent path of the vagus is still open and as a result of afferent impulses reaching the medulla the rate and pressure do not fall as markedly as when afferent impulses are prevented from reaching the medulla. A comparison of tables 2 and 4 will bring this point out.

Again, if the accelerators are sectioned after division of both vagi we find a greater fall in both heart rate and blood pressure than when the accelerators are removed after atropine is injected. This again seems to point to the conclusion that afferent impulses over the vagi tend to keep the central cells in the medulla in check and brings out the relation of afferent to efferent roots in the maintenance of blood pressure. A comparison of tables 6 and 7 will bring this point out.

There is no evidence from these experiments tending to show that afferent impulses over the vagi tend to inhibit the accelerator mechanism after the administration of atropine.

These experiments have also brought out some points on the function of the cardiac nerves. If the vagi are sectioned after the removal of the stellate ganglia, the heart rate and blood pressure increase considerably. The normal action of the vagus then is to maintain a tonic action over blood pressure and heart rate. This fact is brought out still more strikingly in later experiments. If the stellate ganglia are removed and then the cerebral arteries are partially occluded, with the vagus intact, a rise of pressure is obtained as well as an increase in heart rate. The second occlusion, however, results in a rise of pressure which is not as large as the first, while the heart rate also decreases. The third occlusion results in a rise of pressure which is still lower than in the second case, while the heart rate also decreases. Every occlusion after this one is followed by a still smaller rise of pressure and a decreased heart rate. These results indicate that the vagi are concerned with keeping the blood pressure and heart rate down to or sometimes below a level which is to the best interest of the organism. The vagi seem to stand in the way of a rising tide of pressure, and this obstruction may be to the disinterest of the organism. Haldane has pointed out that in the case of the respiratory system the vagi may sometimes act to the disinterest of the organism. The same statement may be made in the case of the cardio-vascular system for sometimes the vagi may stand in the way of a rising pressure when it is to the best interests of the organism to have a high pressure, as in cerebral anemia.

The results obtained on section of the vagi during a state of partial cerebral anemia seem to depend on whether the accelerators are intact or whether they have been previously excised. If the accelerators are intact, section of the vagi is followed by a very high blood pressure. When the normal circulation is restored, the pressure does not, in general, fall below the level maintained at the beginning of the experiment. If, however, the accelerators have been excised preliminary to section of the vagi, partial occlusion of the cerebral arteries results in a rise of pressure which is not very much above the pressure maintained at the beginning of the experiment. Upon restoring the normal circulation, the pressure falls very much below that maintained at the beginning of the experiment. These experiments seem to show that the accelerators are in some way concerned with the maintenance of blood pressure. That they respond to the emergency of a falling pressure is well shown, for in the case where the accelerators are intact the pressure does not fall below normal on restoring the normal circulation. In their absence, however, the pressure does fall below the pressure maintained at the beginning of the experiment when the cerebral circulation is restored.

With the loss of the accelerator mechanism the cardio-vascular mechanism loses an efficient protection against a falling pressure, which would act to the disinterest of the organism. An increased pressure is then due to the accelerator mechanism which works to the best interests of the organism.

The responses of the cardio-vascular system seem to be guided by two antagonistic mechanisms, a cardio-inhibitory nervous mechanism and a cardio-accelerator nervous mechanism, both of which through the algebraic summation of their effects act in the best interests of the organism under ordinary conditions. The first mechanism is concerned with preventing the heart from overwork, and in giving the heart musculature a sufficient period of rest for its metabolic repair. The second mechanism is intimately concerned with the maintenance of a certain level of blood pressure which is to the best interests of the organism.

Friedenthal (25) has shown that animals in which the cardiac nerves have been sectioned exhibit no discomfort when at rest, but are unable to undergo any considerable exertion. This illustrates the tone, accelerator as well as inhibitory, which these nerves maintain over the heart.

The vagus, although the inhibitory nerve to the heart, has been shown in these experiments to contain accelerator fibers. If atropine is

injected, the inhibitory fibers are paralyzed, whereas the accelerator fibers are not affected. If the peripheral end of the cut vagus is now stimulated, the heart rate is accelerated instead of being inhibited. The presence of these fibers in the vagus may also explain in part the increased heart rate which is obtained after a preliminary decrease when a state of partial cerebral anemia is brought about. This is due to a greater resistance of the accelerator fibers to anemia than the inhibitory fibers, hence they will retain their activity after the inhibitory fibers have been made functionless by anemia.

In the experiments in which the adrenals had been ligated preliminary to section of the vagi, the usual result of section of the vagi under these conditions was first a small rise of blood pressure followed by a fall in each case. The heart rate was, however, slightly increased both after ligation of the adrenals and after double vagotomy. What this factor was which caused the accelerated heart after these procedures was not determined.

I wish to express my sincere thanks to Professor Pike for the suggestion of this problem and for his keen interest shown while these experiments were going on.

CONCLUSIONS

1. While the cardio-vascular system may undoubtedly act automatically under conditions which preclude the effect of afferent impulses its normal activity and particularly its response to changing conditions in the organism is not wholly automatic but partly reflex.

2. Afferent impulses over the vagi reflexly affect the central accelerator nervous mechanism.

3. When the cardiac nerves are sectioned, the ultimate heart rate and the ultimate blood pressure depend on whether the vagi or the accelerators have been sectioned first.

4. The accelerators respond to the emergency of a falling blood pressure.

5. The accelerator nerves seem to maintain a tonic action over the heart.

6. The accelerators seem in some way to be concerned with the maintenance of blood pressure.

7. There is a cardio-inhibitory and a cardio-accelerator mechanism, both working, under normal conditions, to the best interests of the organism.

8. There are accelerator fibers in the vagus.

9. Adrenalin seems to be concerned with the rise of blood pressure on section of the vagi under the conditions of these experiments.

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STUDIES ON RENAL TUBULE FUNCTION

I. A Comparison of the Concentration Ratios of Various Urinary Constituents

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Received for publication March 5, 1923

If the "modern theory" (1) of renal secretion is correct, we should find that:

1. All no-threshold bodies should be concentrated by the kidney to the same extent during a given period, i.e., $\frac{\text{urine concentration A}}{\text{plasma concentratio A}} = \frac{\text{urine concentration B}}{\text{plasma concentration B}}$ where A and B are no-threshold bodies.

2. All no-threshold bodies should follow parallel curves of excretion in the urine, with or without diuresis, except in so far as deviations from the parallel can be accounted for by changes in their plasma concentrations.

3. The rate of excretion of any no-threshold body should be proportional to its plasma concentration, provided the rate of glomerular filtration remains constant, or proportional to the rate of glomerular filtration, provided its plasma concentration remains constant.

These papers propose to test the validity of these propositions and to decide on the basis of the data obtained certain points on tubular function now held in question. The present paper deals with the first of the above statements.

The ability of the kidney to concentrate a substance is measured by the ratio of its urine concentration to its plasma concentration. The expression $\frac{\text{urine concentration}}{\text{plasma concentration}}$ has been termed the concentration ratio. Mayrs (2) reports a series of experiments on rabbits comparing the concentration ratios of sulphate, phosphate, urea and creatinine. He finds that creatinine, sulphate and phosphate are concentrated to approximately the same extent, while urea is concentrated less efficiently.

He concludes that creatinine, sulphate and phosphate are no-threshold bodies, while urea is partially reabsorbed. To what extent he is justified in his assumption that, because the three bodies mentioned are concentrated to approximately the same extent when their plasma concentration is at the abnormally high level he employed, their concentration ratios will remain approximately the same with all plasma concentrations and rates of urine flow, will be discussed later.

The experiments reported in this paper compare the concentration ratios of urea, phosphate, sugar in the phlorhizinized dog and chloride. The extension of the observations to sulphate has been withheld, pending the development of a satisfactory method for the determination of sulphate in small amounts of plasma. Creatinine has not been followed because of the present uncertain status of blood creatinine. Behre and Benedict (3) have presented strong evidence that the usual conception of the blood creatinine level is quite erroneous. It is felt that our knowledge of blood creatinine is too incomplete to justify the use of blood creatinine figures as premises in an argument on renal function. The present experiments deal, as did those of Mayrs, with the comparison of single samples of blood and urine. For several reasons it seemed advantageous to follow this method in the first experiments. To find whether sugar in the phlorhizinized animal behaves as a no-threshold body, as Cushny postulates (4); to find whether a typical threshold body, as chloride, can, by previous enrichment of the organism, be made to behave as a no-threshold body; to obtain further data on the variability of the extent to which urea is reabsorbed; to investigate a possible influence of an excess of one constituent on the elimination of another; these were among the reasons for this set of experiments.

Methods. Dogs were used in all experiments except one (exper. 5), where a large male rabbit was used. No regulation of diet or water allowance was carried out previous to the experiments. The method of procedure was simple. A hypodermic injection of 0.016 to 0.048 gram morphine was given and about 30 minutes later the animal put under ether, a tracheal cannula inserted, the bladder catheterized, a cannula from the injecting burette inserted into a femoral vein and the opposite femoral artery exposed for taking samples of blood. Very little ether was required after the completion of the preliminary operations. The animals always remained in good condition. The desired solution (urea, phosphate, etc.) was then injected from a burette at a uniform rate, the time of injection varying in different experiments from 30 to 80 minutes, the bladder completely emptied immediately

at the end of the injection and urine collection started in slender, certified graduated cylinders. The organism was thus enriched with the constituents to be followed, before starting the urine collection. An exception is made in the case of sugar. In practically all cases a good diuresis continued during the collection. The bladder could be completely emptied through the catheter by pressure on the abdominal wall; this was in every case verified by abdominal incision and direct inspection at the close of the experiment. Urine collection was ended in 6 to 11 minutes, during which time two or three blood samples were taken, the mean plasma concentrations of the various constituents during the collection period being calculated from the curves obtained from the analysis figures. The plasma concentration figures given in the tables are the mean figures so obtained. The blood was collected in tubes containing the minimal amount of potassium oxalate necessary to prevent clotting, was immediately centrifuged, the plasma pipetted off and the determinations made immediately or the plasma put in the ice box pending the determinations. Five to 8 cc. of blood sufficed for each sample. Samples on which chloride analyses were to be made were collected under oil. Due haste after collection was exercised in the phosphate determinations. Chlorides were determined by the method of Austin and Van Slyke (5), sugar by Shaffer and Hartmann's method (6), inorganic phosphates by Brigg's modification (7) of the Bell-Doisy method, urea in urine by Fiske's method (8) and urea in plasma at first by Folin and Wu's blood urea method (9), later simply by subjecting 1 cc. plasma to urease action, then adding an equal volume of alcohol, 0.5 gram of equal parts of dry Na_2CO_3 and NaCl and a drop of anti-foam mixture, aerating and Nesslerizing. Duplicates, run on probably half the determinations, always gave good checks. All methods were fairly frequently checked with standard solutions. The percentage of error of the methods ranges from less than 0.5 per cent on the chlorides to probably 3 per cent on the phosphates. The urea N figures on urine have of course been obtained by subtracting the ammonia N from the total N blown over after urease action. The phosphate solutions injected were prepared by bringing Na_2HPO_4 solutions to a pH of 7.4 by the addition of phosphoric acid. The dosage of phlorhizin gave us some concern. Lusk (10) in his review of the literature gives the rule that the subcutaneous injection of 2 gram phlorhizin three times a day will maintain a dog in complete diabetes. On the assumption that Lusk referred to a 10 kilo dog, which may be taken as somewhere near the average size, we have used subcutaneous injections of 1 gram

phlorhizin in 15 to 20 cc. of 1.2 per cent Na_2CO_3 solution per 5 kilos body weight. Further reference to the standardization of the dose will be given in a subsequent communication.

RESULTS. Table 1 gives a comparison of the concentration ratios of chlorides and sugar.

In each experiment an intravenous injection of 50 cc. per kilo per hour of 1.8 per cent NaCl solution was begun 80 minutes before the urine collection was started. This was continued for 60 minutes, when the injection was immediately changed to 30 cc. per kilo per hour of a 10 per cent NaCl solution which was continued for 20 minutes, i.e., until 10 cc. per kilo of a 10 per cent NaCl solution had been

TABLE 1

EXPERIMENT NUMBER	BODY WEIGHT, KILOS	TIME AND SIZE OF MORPHINE DOSE	TIME OF PHLORHIZIN INJECTION. DOSE = 1 GM. PER 5 KILOS	TIME OF URINE COLLECTION	VOLUME OF URINE, CC.	PER CENT NaCl IN PLASMA	PER CENT NaCl IN URINE	CONCENTRATION RATIO OF NaCl	PER CENT SUGAR IN PLASMA	PER CENT SUGAR IN URINE	CONCENTRATION RATIO OF SUGAR	CONCENTRATION RATIO OF NaCl. SUGAR=100
1	11.80	10:45 0.032 gm.	10:45	1:20 to 1:29	132.6	0.980	1.054	1.08	0.129	0.43	3.34	32.3
2	19.50	9:50 0.064 gm.	11:03	12:16 to 12:23	143.0	1.007	1.164	1.16	0.117	0.305	2.61	44.4
3	17.20	9:30 0.048 gm.	11:15	11:52 to 12:00	128.0	0.991	1.088	1.10	0.136	0.52	3.83	28.7

injected. The organism was thus greatly enriched in chloride. In every case the urine collection was started immediately at the end of the injection, or within 1 to 2 minutes. As may be seen in the table, the interval between the phlorhizin administration and the beginning of the urine collection varied from 47 minutes to 2 hours 35 minutes. We may state, however, that the animals in all these experiments were safely phlorhizinized during the urine collection period.

A lengthy discussion of the results shown in table 1 is unnecessary; the conclusion is obvious that the kidney of the phlorhizinized animal concentrates sugar much more efficiently than NaCl, even when the organism has been previously enriched by enormous doses of NaCl. No conclusion can be drawn as to the threshold or no-threshold status

of sugar, since we must find two bodies concentrated to the same extent in order to be in any way justified in regarding them as no-threshold bodies and even then, as we shall see later, conclusions must not be drawn too hastily.

We may next compare the concentration ratios of chloride with those of urea and phosphates.

In experiments 4 and 5 an intravenous injection of 50 cc. per kilo per hour of 1.8 per cent NaCl and 0.5 per cent urea solution was started 80 minutes before beginning the urine collection. This was continued for 1 hour, when the injection was immediately changed to 30 cc. per kilo per hour of a 10 per cent NaCl and 3 per cent urea solution, which was continued for 20 minutes, i.e., until 10 cc. per kilo had been injected. The organism was thus greatly enriched in both urea and chloride. A rabbit was used in experiment 5, chloral-urethane anesthesia. In experiment 12 an intravenous injection of 25 cc. per kilo of a 3 per cent Na_2HPO_4 and 3.6 per cent NaCl solution (pH 7.4) was started at 10:20 and ended at 11:00. Urine collection started at 11:02. The organism was thus greatly enriched in both chloride and inorganic phosphate. In every case the urine collection was started immediately at the end of the injection or within 1 or 2 minutes.

An examination of table 2 shows that the concentration ratio of chloride does not approximate either that of urea or of inorganic phosphate. We are not surprised at the failure of the correspondence of the concentration ratio of chloride with those of urea, inorganic phosphate or of sugar in the phlorhizinized animal. No one has ever claimed that chloride is a no-threshold body. It is interesting, however, to see that it can not be made to behave as one, even when the organism has previously been greatly enriched in chloride.

Let us now compare the concentration ratios of substances which have a stronger claim to being no-threshold bodies, i.e., inorganic phosphate, urea and sugar in the phlorhizinized animal. In table 3 each one of these bodies is compared with each other one in one or more experiments.

In experiment 6 an intravenous injection of 50 cc. per kilo per hour of 0.5 per cent urea, thoughtlessly made up in distilled water, was started 80 minutes before beginning of urine collection and continued for 1 hour. The injection was then immediately changed to 30 cc. per kilo per hour of 3 per cent urea solution in distilled water and continued for 20 minutes. Marked hemolysis and hemoglobinuria resulted from this injection. The comparative failure of diuresis here may be due in part to the hemolysis. Injection in experiment 7 identical in proportion to body weight to that in experiment 6, but urea was made up in 0.7 per cent NaCl solution. No hemolysis or hemoglobinuria. In experiments 9, 13 and 15 an intravenous injection of 25 cc. per kilo of a 3 per cent Na_2HPO_4 and 0.7 per cent NaCl solution (pH 7.4) was started 30 minutes before beginning urine

TABLE 2

EXPERIMENT NUMBER	BODY WEIGHT KILOS	TIME AND SIZE OF MORPHINE DOSE	TIME OF URINE COLLECTION	VOLUME OF URINE, CC.	PER CENT NaCl IN FLASKA	PER CENT NaCl IN URINE	CONCENTRATION RATIO NaCl	MGM. UREA N PER 100 CC. FLASKA	MGM. UREA N PER 100 CC. URINE	CONCENTRATION RATIO UREA N	MGM. INORGANIC P PER 100 CC. FLASKA	MGM. INORGANIC P PER 100 CC. URINE	CONCENTRATION RATIO INORGANIC P	CONCENTRATION RATIO NaCl UREA N = 100	CONCENTRATION RATIO NaCl P = 100
4	11.54	10:03 0.032 gm.	12:20 to 12:30	133.4	0.914	1.411	1.54	35.3	223	6.81				22.7	
5	5.93		11:42 to 11:49	40.8	0.888	1.074	1.21	34.8	203	5.84				20.8	
12	13.20	9:20 0.048 gm.	11:02 to 11:08	88.4	0.819	1.310	1.6				29.4	176	5.98		26.8

TABLE 3

EXPERIMENT NUMBER	BODY WEIGHT, KILOS	TIME AND SIZE OF MORPHINE DOSE	TIME OF PHLORHIZIN INJECTION DOSE = 1 GM. PER 5 KILOS	TIME OF URINE COLLECTION	VOLUME OF URINE, CC.	MGM. UREA N PER 100 CC. PLASMA	MGM. UREA N PER 100 CC. URINE	CONCENTRATION RATIO UREA N	PER CENT SUGAR IN PLASMA	PER CENT SUGAR IN URINE	CONCENTRATION RATIO SUGAR	MGM. INORGANIC P PER 100 CC. PLASMA	MGM. INORGANIC P PER 100 CC. URINE	CONCENTRATION RATIO INORGANIC P	CONCENTRATION RATIO OF LESS CONCENTRATED BODY; GREATER = 100
6	7.61	9:30 0.032 gm.	11:05	11:37 to 11:46	16.2	35.0	673	19.2	0.289	6.16	21.3				90.2
7	8.06	9:15 0.032 gm.	10:55	11:22 to 11:35	31.8	28.7	692	24.1	0.109	2.00	18.3				76.2
9	17.55	9:15 0.064 gm.	10:33	11:01 to 11:11	82.0				0.124	1.09	8.80	46.4	405	8.72	99.1
13	7.27	9:15 0.032 gm.	10:03	10:31 to 10:37	27.6				0.195	1.97	10.1	44.2	427	9.66	95.6
15	9.50	10:00 0.032 gm.	10:40	11:36 to 11:42	35.8				0.128	0.82	6.40	45.8	311	6.80	94.2

10	24.60	9:35 0.064 gm.		11:21 to 11:27	45.2	35.2	124	3.53				39.1	408	10.4	33.8
11	12.95	9:30 0.048 gm.		10:45 to 10:54	52.0	26.7	226	8.48				36.8	410	11.1	76.2

collection and continued for 30 minutes. In experiments 10 and 11 an intravenous injection of 20 cc. per kilo of 3 per cent Na_2HPO_4 and 1.5 per cent urea in a 0.7 per ent NaCl solution (pH 7.4) was started 30 minutes before beginning urine collecion and continued for 30 minutes. In every case the urine collection was begun immediately, or practically so, after completion of injection.

An examination of table 3 shows that sugar in the phlorhizinized dog and inorganic phosphate are concentrated by the kidney to almost exactly the same degree. Urea is concentrated to a much less extent than phosphates,[1] and in one experiment (exper. 6) urea is concentrated less than sugar while in another (exper. 7) urea is concentrated more than sugar.

TABLE 4

	EXPER. 1		EXPER. 2		EXPER. 3		EXPER. 4		EXPER. 5		EXPER. 12	
Concentration ratio	NaCl	Sugar	NaCl	Sugar	NaCl	Sugar	NaCl	Urea	NaCl	Urea	NaCl	P
	1.08	3.34	1.16	2.61	1.10	3.83	1.54	6.81	1.21	5.84	1.60	5.98
Conc. ratio of less concentrated; greater = 100.....	32.3		44.4		28.7		22.7		20.8		26.8	

	EXPER. 6		EXPER. 7		EXPER. 9		EXPER. 13		EXPER. 15		EXPER. 10		EXPER. 11	
Concentration ratio	Urea	Sugar	Urea	Sugar	P	Sugar	P	Sugar	P	Sugar	P	Urea	P	Urea
	19.2	21.3	24.1	18.3	8.72	8.8	9.66	10.1	6.80	6.40	10.4	3.53	11.1	8.48
Conc. ratio of less concentrated; greater = 100...	90.2		76.2		99.1		95.6		94.2		33.8		76.2	

A summary of the results of all the experiments can be seen at a glance in table 4.

DISCUSSION. It is seen that of the four substances examined, chloride, urea, sugar and phosphate, only two, sugar and phosphate, have the same concentration ratio under the conditions of any given experiment. Evidence will be presented in a subsequent communication that the animals in all the phlorhizin experiments may safely be

[1] The unqualified term "phosphate" in these papers will always be understood to refer to the inorganic phosphates and "sugar" refers to sugar in the phlorhizinized animal.

considered as under the maximal influence of phlorhizin, i.e., that the tubular cells were totally rejecting the sugar offered them by the glomerular filtrate, if indeed that is the mechanism involved. This appears as strong evidence in favor of Cushny's view (5) of the action of phlorhizin. As Mayrs says (2), "The concentration ratios of certain very different substances are so nearly the same that it requires a stretch of the imagination to suppose that they are secreted independently of each other. It is simpler to assume that they are concentrated by removal of water and to account for possible slight differences in their concentration ratios by admitting that small quantities of 'no-threshold' bodies may be absorbed." We have seen in the present experiments that the concentration ratios of phosphate and sugar are practically identical. We might infer, then, that when the concentration ratios of urea and sugar are compared they would bear the same relation to each other as do the concentration ratios of urea and phosphate. Actual experiment shows, however, that while phosphate in both cases observed (expers. 10 and 11) is concentrated more efficiently than urea, the latter is in one case (exper. 6) concentrated less efficiently than sugar, as would be expected, but in the other case (exper. 7) is concentrated more efficiently than sugar. If, on the basis of the results of experiments 6, 9, 10, 11, 13 and 15, phosphate and sugar are no-threshold bodies while urea is partially reabsorbed, how are we to explain the results of experiment 7 where, if filtration and reabsorption alone are involved, sugar must be considered as being absorbed to a greater extent than urea? This apparent interchange of the faculty of non-reabsorbability between sugar and urea robs the filtration-reabsorption theory of its simplicity.

Mayrs has neglected the possibility of finding such a discrepancy by merely assuming, since the concentration ratios of sulphate and phosphate are approximately the same and since sulphate and creatinine have approximately the same concentration ratios, that therefore the concentration ratios of creatinine and phosphate are the same and that creatinine will bear the same relation to urea as does sulphate. On the same line of reasoning which, to be sure, appears at first sight logical, we might conclude from our phosphate-sugar comparisons that since the concentration ratios of phosphate and sugar are the same, therefore the relation of urea to phosphate should be the same as its relation to sugar. But, as we have seen above, this is not always the case. The effect of the presence in excess of one substance on the kidney's ability to eliminate the other substance may play a part in

the production of these discrepancies, but the data are not sufficient to warrant any conclusions. While we are quite ready to admit that the close correspondence of the various concentration ratios which do correspond is, to say the least surprising, still we cannot regard it as other than a coincidence or, perhaps, to be explained by the suggestion offered at the end of this discussion. In view of the recent work on blood creatinine, the apparent correspondence of creatinine cannot be regarded as of any significance. And, in view of further observations we have made on the behavior of phosphates and sugar at various plasma concentration levels, some of which are nearer the normal than those which Mayrs has employed and which we have employed in these experiments, and of observations on the behavior of urea at normal plasma levels and with various rates of urine flow, we must ascribe these ratios to some other factor than a filtration-reabsorption process alone. In anticipation of a subsequent communication we may say that evidence will be offered that urea, sugar and phosphate are eliminated, in part at least, through the tubular epithelium and that the correspondence of the concentration ratios of phosphate and sugar breaks down when they are followed through successive periods with varying plasma concentrations.

The correspondence of the ratios observed by Mayrs and in this paper may be due to the fact that abnormally high plasma concentrations of the various constituents were employed. On the view that these constituents are in part eliminated through the tubules we might assume that their great concentration in the plasma would arouse the tubular cells to their maximum secreting activity. The same maximum secreting activity of the cells for sugar is aroused not by a hyperglycemia in these experiments, but by phlorhizin. Regardless of how this maximal activity is aroused, we might suppose that when the tubular cells are excited to secrete two bodies simultaneously at their maximum rate, the maximal concentrating efficiency of the cells for certain substances, as phosphate, sugar and sulphate, might happen to be approximately the same, while even their maximal concentrating efficiency for other bodies, as urea, might to a varying degree fall short of that exhibited toward the first class of bodies. The relation of the respective concentrating efficiencies of the kidney for the different bodies is not, however, an immutable one; thus we see that urea may, on occasion, be concentrated more than sugar.

SUMMARY

1. The concentration ratios of phosphate and sugar under the conditions of the experiments related in this paper are approximately equal.

2. The concentration ratio of chloride is much less than that of phosphate, sugar and urea, and is not raised, but rather lowered by previous enrichment of the organism with chloride. The profuse diuresis, of course, largely explains the lowering of the ratio.

3. The concentration ratio of urea is less than that of phosphate.

4. The concentration ratio of urea is in one experiment less than that of sugar, in another is greater.

5. No conclusions are drawn as to the influence of an excess of any constituent studied on the elimination of any other constituent studied.

6. The lack of justification for considering the correspondence of the concentration ratios of urinary constituents as proof that they are no-threshold bodies, in Cushny's sense, is discussed.

7. A tentative explanation of this correspondence of ratios other than that based on the filtration-reabsorption theory is suggested.

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STUDIES ON RENAL TUBULE FUNCTION

II. A Comparison of the Plasma Concentrations and Rates of Excretion of Various Urinary Constituents

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Received for publication March 5, 1923

In the preceding paper (1) the statement was made that "evidence will be offered that urea, sugar and phosphate are eliminated, in part at least, through the tubular epithelium and that the correspondence of the concentration ratios of phosphate and sugar breaks down when they are followed through successive periods with varying plasma concentrations." It is the purpose of the present paper to present this evidence. The evidence was obtained in the course of an investigation of the second and third propositions stated at the beginning of the preceding paper and in an experiment designed to demonstrate the extent to which a dog was under the influence of phlorhizin at different periods after its administration.

In the preceding paper the concentration ratios of chloride, urea and phosphate were compared with that of sugar in the phlorhizinized dog. It is evident that such a comparison is permissible only on the condition that the animal at the time of the comparison was under the maximal influence of phlorhizin. The mere production of a glycosuria is not sufficient evidence that sugar has been made a no-threshold body or, as Cushny says (2), is being totally rejected by the tubules. In order justifiably to consider sugar as a no-threshold body we must confine our observations to a period when the kidney is eliminating sugar with the greatest possible efficiency. We must, then, discover at what time after subcutaneous phlorhizin injection the kidney eliminates sugar most efficiently; i.e., according to Cushny's conception, at what time the tubules totally reject sugar. Knopf (3) maintained a D:N ratio of 4.2 for 4 days in a 24-kilo dog by the subcutaneous injection of 2 grams phlorhizin every 8 hours. We are interested, however, in finding how soon the condition of complete rejection of sugar is established and whether or not it may be considered as completely maintained during

the time periods used in these experiments. The following experiment deals with this question.

Experiment 1. A dose of 1 gram phlorhizin in 20 cc. of 1.2 per cent Na_2CO_3 solution per 5 kilos body weight was given subcutaneously to a dog weighing 4.6 kilos which had been on an unregulated diet. One hour previously 0.016 gram morphine had been given subcutaneously. The animal was anesthetized with ether, a tracheal cannula inserted and the bladder catheterized. At the time of the phlorhizin administration an injection into the right femoral vein of 20 cc. per kilo per hour of 0.7 per cent NaCl solution was started and was continued throughout the experiment, to maintain a moderate diuresis. Fifteen minutes after the phlorhizin administration the bladder was emptied and urine collection started. The urine collections were made during 15 minute periods for the first three samples, a blood sample being taken from the left femoral artery at the middle of each urine collection period. The next three samples were taken at 30-minute intervals and the last after an interval of 60 minutes. Sugar determinations were immediately made on the urine and plasma samples. The results are seen in table 1.

TABLE 1

SAMPLE	PLASMA PER CENT SUGAR	URINE PER CENT SUGAR	CONC. RATIO OF SUGAR	URINE CC. PER HOUR	GRAMS SUGAR PER HOUR	PROPORTION OF SUGAR ELIMINATED TO AMOUNT AVAILABLE
1	0.132	4.53	34.3	53.6	2.43	18.4
2	0.104	4.88	46.9	41.6	2.03	19.5
3	0.103	5.25	51.0	48.0	2.52	24.5
4	0.090	6.13	68.1	32.4	1.99	22.1
5	0.081	6.20	76.6	32.4	2.01	24.8
6	0.073	4.70	64.4	38.0	1.79	24.5
7	0.046	2.73	58.0	51.2	1.40	30.4

One might ask what is to be taken as the criterion of the kidneys' maximal efficiency in eliminating sugar. Is it the period when the concentration ratio is highest, or when the most sugar per unit time is being eliminated? Neither is necessarily the case. Let us say that it is the period during which the kidney eliminates the most sugar in proportion to the amount available for elimination. Since there is no reason to assume any significant variations in the blood volume flow of the kidney during the experiment, the amount available may be considered as being proportional to the plasma sugar concentration. Thus in period 1 the available sugar is 132, in period 2, 103, etc. We may now calculate for each period the amount of sugar eliminated in proportion to the amount available. Thus, in period 1, with 132 arbitrary units

of sugar available per unit time, we find 2.43 grams of sugar eliminated per unit time. Dividing the second term by the first we find that for each unit of available sugar the kidney eliminated 18.4 mgm. of sugar. The figures so obtained for each period will be proportional to the amount of sugar eliminated from each unit of sugar available for elimination; in other words, we may take the figures so obtained as representing the proportion of sugar eliminated to that available. The figures in the right hand column of the table represent the proportions so calculated. We thus see that in period 7 the kidney is eliminating the largest amount of sugar in proportion to the amount available, which does not coincide either with the period of highest concentration ratio (period 5), or the period during which the greatest absolute amount per unit time was being eliminated (period 3). One might object that, on the basis of the filtration-reabsorption theory, we should try to find, not the period in which the greatest amount is eliminated in proportion to the amount available as indicated by the plasma concentration, but the period in which the greatest amount is eliminated in proportion to the amount filtered through the glomeruli. In other words, that the amount filtered rather than the amount brought by the plasma is the amount truly available. An adequate consideration of the points raised here would take us too far afield, and there are in fact no data at present available for a final decision of the question. That, however, is not necessary for our present purposes. We believe the data are such as to justify the conclusion that the animal is safely phlorhizinized within 30 minutes after the subcutaneous injection and that this state is maintained for several hours. In other words, that sugar may be regarded as a no-threshold body during the periods of the experiments reported in the present papers, if it can ever justifiably be so regarded.

We may now proceed to a consideration of the validity of the propositions that:

1. All no-threshold bodies should follow parallel curves of excretion in the urine, with or without diuresis, except in so far as deviations from the parallel can be accounted for by changes in their plasma concentrations.

2. The rate of excretion of any no-threshold body should be proportional to its plasma concentration provided the rate of glomerular filtration remains constant, or proportional to the rate of glomerular filtration provided its plasma concentration remains constant.

The experiments reported in the preceding paper, comparing the concentration ratios of various bodies, showed that of the bodies

followed, only phosphate and sugar had the same concentration ratios. These two substances are therefore taken as having passed the first test for no-threshold bodies, and are now to be subjected to the test stated in proposition 1 above. This was done in the following experiment.

Experiment 2. In this experiment the rates of excretion and the plasma concentrations of phosphate and sugar were followed in a phlorhizinized dog with the plasma concentrations approximately normal. A dog of 12.27 kilos was given 0.048 gram morphine at 9:00. Put under ether, tracheal cannula inserted and 1 gram phlorhizin per 5 kilos body weight given subcutaneously at 10:15. Injection of 20 cc. per kilo per hour of 1.8 per cent NaCl solution into right femoral vein started at 10:20 and ended at 10:35. This was to promote diuresis. First urine collection started at 10:35 and collection periods in all cases were 15 minutes. Since, as may be seen in the table, the urine flow during the first period was not particularly strong, an injection of 40 cc. per kilo per hour of 1.8 per cent NaCl

TABLE 2

SAMPLE	PLASMA PER CENT SUGAR	URINE PER CENT SUGAR	CONC. RATIO SUGAR	GRAMS SUGAR PER HOUR	PLASMA MGM. P PER 100 cc.	URINE MGM. P PER 100 cc.	CONC. RATIO P	MGM. P PER HOUR	URINE CC. PER HOUR	CONC. RATIO OF P SUGAR = 100
1	0.332	3.37	10.1	1.97	3.88	6.67	1.72	3.95	58.4	17.0
2	0.305	7.10	23.3	4.85	3.91	2.44	0.67	1.41	68.0	2.9
3	0.312	7.00	22.5	2.97	3.96	2.08	0.52	0.88	42.4	2.3
4	0.222	6.80	30.6	2.94	3.91	2.08	0.53	0.90	43.2	1.7
5	0.218	1.24	5.70	3.44	3.85	0.5–		Trace	277.6	
6	0.195	0.56	2.87	2.58	3.09	0.5–		Trace	460	

solution was started at 10:50, i.e., at beginning of second collection period. Since it was our intention to follow the kidneys' behavior at varying rates of urine flow, the injection rate was increased to 60 cc. per kilo per hour at 11:05, i.e., at beginning of third collection period. And during the fourth, fifth and sixth periods there were carried on injections of 20, 40 and 60 cc., respectively, per kilo per hour of 7.2 per cent NaCl solution. Each urine collection period was of 15 minutes duration and a blood sample was taken from the left femoral artery in the middle of each period. Results are seen in table 2.

A glance at the table shows that the results of this experiment are in marked contrast to those reported in the preceding paper. There the correspondence of the concentration ratios of phosphate and sugar in single samples was almost exact. Here we see that when these bodies are followed through several periods with varying plasma concentrations and rates of urine flow, the correspondence breaks down completely. With a normal plasma phosphate level, at no time does the concen-

tration ratio of phosphate correspond to that of sugar. It varies from 17 per cent of that of sugar to 1.7 per cent and in the last two periods cannot be determined at all. This is in absolute contradiction to the conclusions drawn by Mayrs (4). He compared the concentration ratio of sulphate to that of phosphate and creatinine, in single samples. He found that, under the conditions of his experiments, the concentration ratios of these bodies were approximately the same and concludes from this that they are no-threshold bodies, or practically so, and are concentrated to the same extent. It does not at all follow that, because the concentration ratios of two bodies correspond at abnormally high plasma levels, they will also correspond under normal conditions, or that the bodies will follow parallel curves of excretion, except in so far as deviations from the parallel can be accounted for by changes in their plasma concentrations. This is clearly shown in the present experiment.

Marshall (5) reports two experiments in which he followed the rate of excretion and the plasma concentrations of chloride, urea and creatinine and concludes "that changes in the plasma can not be responsible for the changes in the urine." As a result of further experiments (6), (7) in which the rate of excretion of various urinary constituents by a denervated kidney was followed, the rate of excretion from the opposite normal kidney being followed in lieu of plasma concentration observations, and of conclusions based on the work of Behre and Benedict (8), Nash and Benedict (9) and Marshall and Vickers (10), Marshall and his associates (7) conclude that "The passage of the non-colloid constituents of the plasma takes place through the glomerulus by filtration. Water, chlorides, carbonates, (possibly urea, sulphates and phosphates) are reabsorbed during the passage of the filtrate along the tubules. Phenolsulphonephthalein (possibly also urea, sulphates and phosphates) are added to the glomerular filtrate by secretion by certain parts of the tubule, while creatinine and ammonia are added by the tubules after being formed in the renal cells." This leaves open the question as to whether the discrepancy in the concentration ratios of, let us say, urea and phosphate, or of sugar and phosphate can be accounted for on the basis of a more complete reabsorption of the less concentrated body, or whether it is necessary to invoke tubular secretion to account for the higher concentration of the more concentrated body. The results of this experiment and of the three which are to follow throw light on the question of the necessity of invoking tubular secretion.

To restate the issue briefly, there is no question but that the conception of filtration with complete, or almost complete, failure of reabsorption of certain bodies, urea, phosphate and sugar in the phlorhizinized animal, must be abandoned. Creatinine is no longer even to be considered, as Marshall, on the basis of Behre and Benedict's work, has already pointed out. The question, as left by Marshall, is, "Can we explain the lesser concentration ratio of, let us say, urea, as compared with that of phosphate, by a more complete reabsorption of urea, or must we invoke a tubular secretion of phosphate?" A consideration of the results of this experiment and of the three to follow answers the question of tubular secretion in the affirmative. We may say that the experiments reported in this paper are typical representatives of numerous similar experiments which have been performed during this investigation. Let us consider the phosphate figures in the present experiment. The rate of glomerular filtration in the second period (see table 2), with a urine flow of 68 cc. per hour, is greater than, certainly as great as, the rate of glomerular filtration in the first period, with a urine flow of 58.4 cc. per hour. If we assume that the rate of glomerular filtration in the second period is merely as great as that in the first, then, on the filtration-reabsorption basis, with phosphate a no-threshold body, the amount of phosphate excreted per hour in the second period should bear the same relation to the amount excreted per hour in the first period as does the plasma phosphate concentration of the second period to that of the first. The plasma phosphate concentration of the second period is 101 per cent of that of the first. We should expect, therefore, to find 101 per cent as much phosphate excreted per hour in the second period as in the first. But we actually find only 35.7 per cent as much phosphate excreted per hour in the second period as in the first. This, of course, eliminates phosphate from the class of no-threshold bodies, but still leaves open the question as to whether the failure of appearance of the expected amount of phosphate in the second period is to be accounted for on the basis of a more complete tubular reabsorption of phosphate in the second period as compared with the first, or on the basis of a less active tubular secretion of phosphate in the second period as compared with the first. But a glance at the rate of urine flow will show that it is unreasonable to consider phosphate as being more completely absorbed in the second period than in the first. It is inconceivable that phosphate should be more completely absorbed when the filtrate is being washed down the tubules at a rate sufficient to give 68 cc. of urine per hour than when it

is being washed down at a rate sufficient to give 58.4 cc. of urine per hour. The only possible explanation is that at least part of the phosphate is being actively secreted by the tubules and that this secretion is more intense during the first period than during the second.

It will be noted that after the first period the concentration of phosphate in the urine is actually less than that in the plasma. This observation alone would eliminate phosphate from the class of no-threshold bodies.

A consideration of three experiments which deal with an investigation of proposition 2 stated above confirms the conception of a process of tubular secretion of phosphate and extends it to sugar and urea. We may first consider the evidence for tubular secretion of sugar. It is seen that the experiment recorded in table 1 bears on this question. If the mechanism of kidney action is merely one of filtration and reabsorption and if sugar in the phlorhizinized animal is a no-threshold body, then the amounts of sugar eliminated per unit time in any two or more equal periods should stand in the same relation as do the plasma concentrations in those periods, provided the rate of glomerular filtration remains constant. It will be seen that the rate of urine flow was maintained fairly constant throughout the experiment. There is, therefore, no reason to assume any great changes in the rate of glomerular filtration. Let us compare periods 2 and 6. If the rate of filtration is the same, then, on the filtration-reabsorption basis, there should be $\frac{0.073}{0.104}$ or 70 per cent as much sugar per unit time eliminated in period 6 as in period 2. There are 2.03 grams of sugar per hour eliminated in period 2. In period 6 we should expect, then, 70 per cent of 2.03 or 1.42 grams per hour. We find, however, that there are actually 1.79 grams per hour eliminated in period 6, or 26 per cent more than the amount expected. One can not say that the extra 26 per cent in period 6 is due to a more rapid filtration, with the carrying through of more sugar. For there is no reason to assume that filtration is more rapid with a urine flow of 38 cc. per hour than with a flow of 41.6 cc. per hour. Neither can one say that the increase over the expected amount in period 6 is due to the fact that a reabsorption of sugar was taking place to a greater extent in period 2 than in period 6. For there is no reason to assume a more complete reabsorption when the filtrate is presumably washed down more rapidly, certainly with as great rapidity. We see no possible explanation of the facts other than a tubular secretion of sugar which is more active, in proportion to the amount of sugar available, in period 6 than in period 2.

Another experiment in which phosphate was followed at various plasma phosphate levels confirms the idea of tubular secretion of phosphate.

Experiment 3. Body weight of dog 17.27 kilos. Experimental conditions were as in previous experiments. All urine collection periods 15 minutes. Two blood samples taken during each urine collection period, average figures given in tables. Results in table 3.

It is seen by the argument applied above, that a tubular secretion of phosphate must be invoked. For on the filtration-reabsorption basis we should expect at least $\frac{7.1}{1.04}$ or 68 per cent as much phosphate to be excreted in the last period as in the first. But we find only 37.2 per cent as much phosphate excreted in the last period as in the first. This can not be explained on the basis of a lower rate of filtration in the last

TABLE 3

SAMPLE	PLASMA MGM. P PER 100 CC.	URINE MGM. P PER 100 CC.	URINE CC. PER HOUR	MGM. P PER HOUR
1	10.4	762	60.0	457
2	8.9	426	64.0	273
3	7.4	241	101.6	245
4	7.1	156	108.8	170

period, for the rate of urine flow here is 108.8 cc. per hour, as compared with 60 cc. per hour in the first period. Neither can we assume a more complete reabsorption of phosphate in the last period, since the filtration rate is greater. We can explain it only on the basis of a tubular secretion of phosphate, which was in this case more active in the first period than in the last.

It will be noted that the activity of the tubular secretion is not always greatest at the beginning of the experiment, nor is it always at the end. In the case of sugar, in the experiment cited in table 1, the secreting activity for sugar was greater at the end than at the beginning, while this is reversed for phosphate in the present experiment. Just what the conditions are which determine the activity of the tubular secretion, is not clear. Apparently there is no constant relation to the plasma concentration of the constituent in question, or to the organism's presumed need for the constituent, although the fact that the concentration ratios of phosphate and sugar are almost the same when the secreting activity of the cells for these substances has presumably been maximally aroused

is suggested as evidence that there may be a limiting factor common to their respective secretory mechanisms.

The next experiment deals with the behavior of urea.

Experiment 4. In this experiment normal plasma levels of urea were employed and the rate of urine flow varied by the injection of NaCl solutions of increasing strength and at increasing rates. Body weight of dog, 10.70 kilos. All urine collection periods 15 minutes, blood sample taken in middle of each period. Experimental conditions as in previous experiments. Results in table 4.

With an increase in rate of urine flow in the last period, as compared with the first, of 678 per cent one is certainly safe in assuming an increase in rate of glomerular filtration of at least 100 per cent. On the assumption of 100 per cent increase in glomerular filtration rate one would expect a corresponding increase in urea excretion, since the plasma concentration of urea is practically unchanged. We find, however, an increase in urea excretion of only 4 per cent. The failure of the expected

TABLE 4

SAMPLE	PLASMA MGM. UREA N PER 100 CC.	URINE MGM. UREA N PER 100 CC.	URINE CC. PER HOUR	MGM. UREA N PER HOUR
1	16.2	402	36.0	145
2	16.0	362	44.2	160
3	16.1	176	88.4	155
4	15.8	62	244.0	151

increase can not be explained as due to a more complete reabsorption of urea in the last period, for it is inconceivable that urea, or any other body, would be more completely absorbed during a period of very rapid filtration rate than during a period of moderate filtration rate. We are forced to the assumption of a tubular secretion of urea, which in this experiment is of almost constant intensity.

DISCUSSION. It will be noted that the present experiments do not tell us what proportion of the urea, sugar and phosphate eliminated is due to glomerular filtration and to tubular secretion, respectively. There is no reason to suppose that part does not come through the glomeruli. The evidence in favor of glomerular filtration is so convincing that there seem to be no good grounds for questioning it and there is no reason to consider the glomerular membrane as impermeable to sugar and phosphate. Loewi's objections (11) are adequately answered by the work of Cushny (12) who has shown that all the non-colloid constituents of ox serum, except Ca and Mg, are in simple solu-

tion and can be filtered off at a pressure of 150 mm. Hg. Furthermore, Wearn (13) has demonstrated sugar in the glomerular capsule of the frog's kidney at a time when the bladder urine was free from sugar. The data at present available, then, enable us to say that, in the conditions of these experiments, part of the urea, sugar and phosphate was eliminated through the glomeruli and part through the tubules, but do not permit us to judge of how this division is proportioned.

Under normal circumstances sugar is reabsorbed by the tubules. Are we to assume that in the phlorhizinized animal this process continues and the tubules are simultaneously secreting and absorbing sugar? There is no reason to assume this. All the known facts can be explained by assuming that phlorhizin both inhibits the tubules' power to absorb sugar and excites them to secrete sugar. No mystical "vitalistic" process need be invoked in this procedure. All physiological phenomena are "vital;" that does not mean that they are not due to the orderly operation of physical and chemical laws. There is no justification in labeling a hypothesis "vitalistic" merely because the facts on which it is founded can not be explained with our present incomplete knowledge. It is quite true that the "modern theory," by its very simplicity falls readily into a "mechanical scheme." But if certain facts are presented which can not be reconciled with this theory and which necessitate the conception of tubular secretion, there is no reason why tubular secretion may not be regarded as just as "mechanical" a process as filtration and reabsorption, if only we understood the laws by which the mechanism operates.

Marshall lists creatinine, ammonia and phenolsulphonephthalein as the substances which are certainly eliminated by the tubules and states that "possibly also urea, sulphates and phosphates are added to the glomerular filtrate by secretion by certain parts of the tubule." We would add urea, phosphate and sugar in the phlorhizinized dog to the list of substances which are certainly added to the glomerular filtrate by tubular secretion. This does not mean that these substances are not also in part filtered through the glomeruli. What fraction comes by each route it is at present impossible to say.

SUMMARY

1. The correspondence of the concentration ratio of sugar in the phlorhizinized dog with that of inorganic phosphate is not maintained with varying plasma levels of these constituents and with varying rates of urine flow.

2. Evidence is presented that urea, sugar in the phlorhizinized dog and inorganic phosphate are in part secreted by the tubular cells.

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